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Biobeds for Environmental Protection from Pesticide Use—A Review

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Biobeds originated in Sweden in response to the need for simple and effective methods to minimize environmental contamination from pesticide use, especially when filling spraying equipment, a typical point source of contamination. The biobed system has attracted attention in several countries, where work is being conducted to adapt it to local conditions and applications. As a consequence, the biobed system has been more or less modified and sometimes renamed, for example, as biomassbed in Italy, biofilter in Belgium, and Phytobac and biobac in France. The effectiveness and simplicity of the biobed also make it suitable for use in developing countries, and different adaptations of the biobed concept now exist in, for instance, Peru, Guatemala, and Ecuador. When the modification of the biobed includes an intention to use it for retention and degradation of pesticides in sprayer washings, the construction has to be adapted to, for example, lined biobeds to ensure that no pesticide leaching will occur. Replacement of some of the original materials in the Swedish biomixture (straw, peat, and soil) can also change the performance of the system, for instance, the amount, activity, and composition of the microbial community that develops. This review presents the state of the art of biobeds and similar systems in Sweden and worldwide and identifies future research needs. Factors affecting the efficiency of biobeds in terms of degradation and retention of pesticides are discussed, with particular emphasis on the microbial processes involved.

1. INTRODUCTION

Unsatisfactory management of pesticides and other chemicals can give rise to residues in surface waters and groundwater and in large volumes of soil. One source of contamination is the use of pesticides in agriculture, and it is important to identify on-farm practices for pesticide handling and use that pose major risks for contamination. Three critical steps are usually involved (**Figure 1**): (1) pouring of pesticide concentrates into the spray tank and their dilution, (2) spraying of pesticides in the field, and (3) management of pesticide residues left on the inside and outside (retained on the outer walls) of the spray tank.

If pesticides are used at the recommended doses and applied using modern techniques according to good farming practices, the risk of environmental contamination from spraying in the field (step 2) is small. However, the risk is greater in steps 1 and 3, which can give rise to point source contamination. Step 1 contributes small spills but at high pesticide concentrations, since concentrates are handled. Step 3 can contribute larger volumes but with lower concentrations from the remaining diluted pesticide in the tank and from washing and consequent dilution of the pesticides retained on the outer walls of the tank.

It could be argued that the risk for contamination from step 1 is small because of the generally small volume of the spill. However, a few drops of a pesticide concentrate can easily contain 1 g of the active substance. If these drops form a spot 1 dm^2 in area on the ground, the final dose is 1 g dm^{-2}, which is equivalent to 1 ton ha^{-1}. Normal pesticide doses for modern products are in the order of kg ha^{-1} or g ha^{-1}. The risk of contamination is therefore obvious (**Figure 2**).

Moreover, spraying equipment is normally filled in the same place on the farm every time, often in the farmyard near a water source and where the topsoil has generally been removed and replaced with a layer of gravel and sand. The poor degradation

Figure 1. Pesticide handling at farms.

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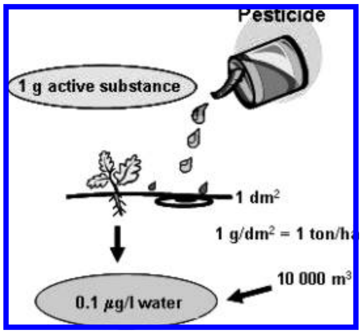

Figure 2. One gram of active substance can easily be accidentally spilled. If it forms a spot of 1 dm^2, the applied dose corresponds to 1 ton ha^{-1}. Dilution of this amount to 0.1 μg L^{-1}, which is the maximum accepted concentration in drinking water in the European Union, requires 10000 m^3 of water. Adapted from ref *8*.

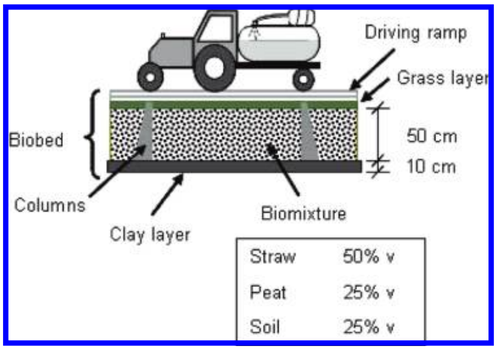

Figure 3. The biobed is a construction intended to retain and degrade spillage of pesticides. It consists of a biomixture (straw, peat, and soil), a clay layer, and a grass layer. A driving ramp is needed for parking the spraying equipment over the biobed (*13*).

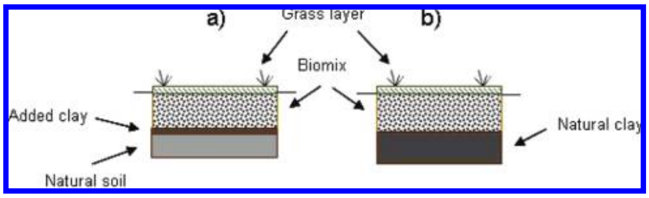

Figure 4. Unlined biobed with (**a**) an added or (**b**) a natural clay layer.

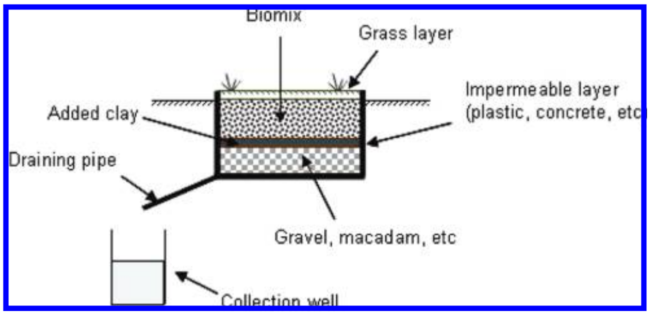

Figure 5. The lined biobed is isolated with an impermeable layer that allows collection of drainage water in a well.

and sorption capacity of these materials increase the risk of leaching of the pesticides. High concentrations of pesticide residues have in fact been found at such sites, and Danish (*1–3*), German (*4–6*), and Swedish (*7*) studies have shown that such point sources of pesticides are one of the dominant causes of pesticide pollution.

A low-cost system known as the biobed can minimize the risks of pollution when filling and storing the sprayer. Biobeds originated in Sweden, but several other systems, based on the principles of the biobed, have now been developed and implemented in many countries, where they have often been renamed, for example, biofilter, biomassbed, Phytobac, and biobac. A recent review paper dealing with such on-farm bioremediation systems (*9*) provides an overview of three systems in particular: biobeds, Phytobac, and biofilters. Aspects such as substrate, design, operation, and functionality of the systems and factors affecting the behavior of the pesticides are discussed.

In this review, we present the state of the art of biobeds and similar systems in Sweden and worldwide and discuss factors affecting the efficiency of the biobeds, that is, degradation and retention of pesticides, with particular emphasis on the microbial processes involved.

2. THE BIOBED—A SWEDISH CONTRIBUTION TO ENVIRONMENTAL PROTECTION FROM PESTICIDE POLLUTION

2.1. What Is a Biobed? The original Swedish biobed is a simple and cheap construction intended to collect and degrade spills of pesticides on farms (*10, 11*). It consists of three components in a 60 cm deep pit in the ground (**Figure 3**): (i) a clay layer at the bottom (10 cm); (ii) a biomixture or biomix of straw, peat, and soil (50:25:25 vol %) filling the remaining 50 cm depth; and (iii) a grass layer covering the surface. The biobed is also equipped with a ramp to allow the sprayer to be driven and parked over the biobed. Greenhouse biobeds also have been developed, and their design varies from that of farm biobeds (*12*). However, the greenhouse biobeds are not discussed further in this review.

2.2. Types of Biobeds. Depending on whether or not the bottom of the biobed is isolated from the environment, there are two types of biobeds: the unlined biobed and the lined biobed.

2.2.1. Unlined Biobed. The unlined biobed has no impermeable synthetic layer that isolates it from the ground. The original Swedish-designed biobed belongs to this group. In many cases, a natural clay layer is present at the bottom of the biobed pit. If this is not the case, a clay layer is added. There is no collection of drainage water in this system (**Figure 4**).

2.2.2. Lined Biobed. The lined biobed resembles the original Swedish biobed but is lined by a synthetic impermeable layer (plastic, concrete, tarpaulin, etc.) that isolates it from the ground. This design allows the collection of drainage water in special wells that are built at the side of the biobed (**Figure 5**). Drainage layers (gravel, macadam, or sand) are usually placed below the clay. This design is in use in the United Kingdom.

2.3. Components of the Swedish Biobed. The efficiency of retention and degradation of pesticides depends on each of the components of the biobed: the clay layer, the biomixture, and the grass layer.

2.3.1. The Clay Layer. Clay, with its low permeability and high sorption capacity, is used as an impermeable layer to decrease the water flow downward and to increase the pesticide retention time in the biobed. A prerequisite for a well-functioning clay layer is that the clay material is wet and swollen to avoid formation of cracks and preferential flow processes. However, preferential flow paths can be formed if the clay dries out, for example, by evapotranspiration or by a break in the capillary water flow. Such conditions can arise when a drainage layer is placed below the clay, for example, as in lined biobeds. This topic is discussed in section 4.2.

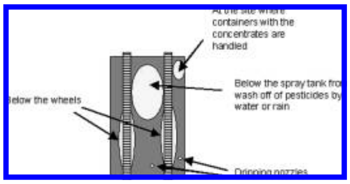

Figure 6. Pesticide spillage pattern in biobeds. The spots represent the damage to the grass layer caused by herbicide spills (*13*).

Figure 7. Different models of biobeds at Swedish farms (*10*).

2.3.2. The Biomixture. The biomixture should have the ability to retain and degrade pesticides. To achieve this, the biomixture should have a good absorption capacity and a high microbial activity. Both capacities are affected by the composition, homogeneity, age, moisture, and temperature of the mixture.

The original Swedish biomixture (OSB) consists of straw, peat, and soil in the proportions 50:25:25 vol %. Each component of the biomixture plays an important role in the efficiency of retention and degradation of the pesticides.

The straw is the main substrate for pesticide degradation and microbial activity, especially from lignin-degrading fungi (such as white rot fungi), which produce phenoloxidases (peroxidases and laccases). The broad specificity of these enzymes makes them suitable for degradation of mixtures of pesticides. For example, the dissipation of most of the pesticides in a mixture has been correlated with phenoloxidase activity and/or basal respiration, with both activities correlated with the levels of straw (*14*). Moreover, the degradation of single pesticides by white rot fungi/peroxidases has been demonstrated in several studies (*15–19*). Therefore, a high amount of straw in the biomixture is recommended, although in practice not more than 50 vol % due to the requirement to achieve a homogeneous mixture (*11, 14*).

The soil provides sorption capacity and should be rich in humus and have a clay content that promotes microbial activity (*20*). However, the clay content should not be so high that it decreases the bioavailability of the pesticides (*21*) or makes it difficult to achieve a homogeneous biomixture. The soil is also an important source of pesticide-degrading microorganisms, especially bacteria with the ability to metabolically degrade such chemicals. However, because of the high C/N ratio and the low pH of the biomixture (to favor lignin-degrading fungi), these processes may be restricted (*14*). Nevertheless, the presence of soil bacteria can enhance the extent of pesticide degradation, as has been observed for other organic pollutants, for example, fungal transformation followed by bacterial degradation of the more polar metabolites enhances the degradation of benzo(*a*)pyrene (*22*).

The peat in the biomixture contributes to sorption capacity, moisture control, and also abiotic degradation of pesticides, as observed for terbuthylazine (*14*). It also decreases the pH of the biomixture as shown by the significant negative correlation between the pH and the volume of peat in the biomixture (*14*). A high peat content thus gives a low pH favorable for fungi, but levels of 50 vol % or more decrease microbial activity, perhaps by giving a too low pH and water availability. Therefore, a peat level of 25 vol % is recommended, which gives a final pH of about 5.9, suitable for lignin-degrading fungi (*14*). An important factor that often activates the fungal lignin-degrading system is nutrient limitation, especially nitrogen deficiency; therefore, the addition of nutrients to the biomixture is not recommended.

2.3.3. The Grass Layer. The grass layer contributes toward increasing the efficiency of the biobed, especially that of the upper parts where most of the pesticides are retained and degraded. It also helps to regulate the moisture of the biobed by creating an upward transport of water and, in addition, can produce root exudates to support cometabolic processes. Peroxidases also can be exudated by grass roots (*23*).

The absence of the grass layer gives poor evapotranspiration and can generate a hydrophobic crust at the top of the biobed, with decreased microbial activity. Moreover, a crust also promotes the drainage of water to the bottom of the biobed by preferential flow (*24–26*), increasing the risk of pesticide leaching. The moisture in systems without a grass layer and without peat in the biomixture may thus be difficult to balance (*26*). Swedish experiences also show that it is important that the biobed is not placed in a barn or under a roof, since this can restrict the development of the grass layer by poor evapotranspiration or drying of the biomix.

The grass layer is also an excellent demonstration tool since it reveals herbicide spillages in particular. Grass damage is often observed at sites where the concentrated pesticides are handled, below the middle of the sprayer tank from surface runoff, below pesticide-contaminated wheels, and below faulty tubings and leaking nozzles (**Figure 6**).

2.4. Biobeds in Practical Use in Sweden.

Biobeds have been in use in Sweden since 1993 when the first prototypes were built and studied (*11, 27*). They have proven to be an effective, simple, and inexpensive way to mitigate leaching of pesticides to surface waters and groundwater (*28*). Moreover, the use of local materials, natural microorganisms, and quick and inexpensive procedures and equipment has contributed to their uptake by farmers and environmental authorities. Several models have been built by farmers, who often reuse old building materials from the farm (**Figure 7**). At present, it is estimated that there are more than 1500 biobeds in use in Sweden. In addition, the biobed is often used for safe diesel refuelling of tractors and other machinery.

Studies to evaluate the performance of the biobeds under Swedish conditions (*10, 11*) show that the pesticides are mainly retained in the upper part of the biobed and that most of them are degraded within 1 year. In the lower levels of the biomixture, concentrations are generally near or below the detection limit, suggesting limited transport to the bottom. The highest pesticide levels are observed during the spraying season, that is, when they are most intensively used.

Because of the degradation of the straw in particular, the height of the biomixture decreases by approximately 10 cm per year under the conditions in southern Swedish. The lost volume is replaced by adding fresh biomixture every year before the spraying season. However, the carbon content in the core of the biomixture decreases with time to levels similar to those found in agricultural soils. The whole biomixture therefore needs to be regularly replaced with fresh material. It is recommended that this is done every 6−8 years under Swedish conditions. The material removed can contain small amounts of pesticide residues, either from pesticides used just before removal of the biomixture or from pesticides that are slowly degraded (for example, deltamethrin and fenpropimorph, unpublished data). Therefore, it is recommended that the material is composted for 1 year, which has proven sufficient to decrease the levels of the pesticide residues to below the limit of detection (*10*).

The highest temperatures in the biobeds are observed during the summer, and the levels depend on where the biobed is located (*10*). Maximum temperatures of 17−20 °C have been observed in southern Sweden and 15 °C in the north. During winter, the temperature fluctuates between 2 and 4 °C in the south, while in the north, the central parts of the biobed tend to freeze.

The moisture in a biobed is of critical importance since it affects oxygen availability and microbial activity and since oversaturation of the biobed can give pesticide leaching. Oversaturation can occur, for example, if the sprayer is washed on the biobed. Therefore, the biobeds in Sweden are intended to be used exclusively for handling of pesticide concentrates and for storing the spraying equipment when not in use during the vegetation period. Washing of the sprayer, as well as of the tractor, should be performed in the field.

Rainwater is allowed into the biobed. However, it is important that the biobed is not placed in a low-lying part of the farmyard to prevent runoff from a large area around the bed entering it.

3. BIOBEDS IN THE WORLD

The biobed has generated interest in other countries (e.g., England, Belgium, Italy, France, Peru, and Guatemala), and its implementation has sometimes led to modifications of the original biobed design into what are renamed biofilters, biomassbed, Phytobac, biobac, and biotables (**Figure 8**).

The introduction of the biobed concept in a new country involves intensive research to adapt it to the conditions, practices, and needs of the particular country. In this section, such research and pilot/field-scale studies in some countries are reviewed.

3.1. Biobeds in the United Kingdom. Several studies have been performed by Fogg et al. to adapt the biobed concept to the agricultural practices and climatic conditions of the United Kingdom (*24, 34–36*). One special issue was to address both the small drips and spills from the normal mixing procedure and the larger volumes of water from tank and equipment washing, which can lead to significant water contamination if not disposed of correctly. The studies were therefore performed to determine the degradability of a range of pesticides in biobeds under conditions that are likely to occur in the United Kingdom and with large water volumes and involved a combination of laboratory, semifield, and field studies. The adaptation of the biobed led to two major changes: (i) isolation of the biobed system from the ground by using an impermeable synthetic liner, that is, use of lined biobeds and (ii) modification of the depth of the biobed from 0.6 m in the Swedish design to 1−1.5 m in the English version to increase the retention time of the pesticides in the bed.

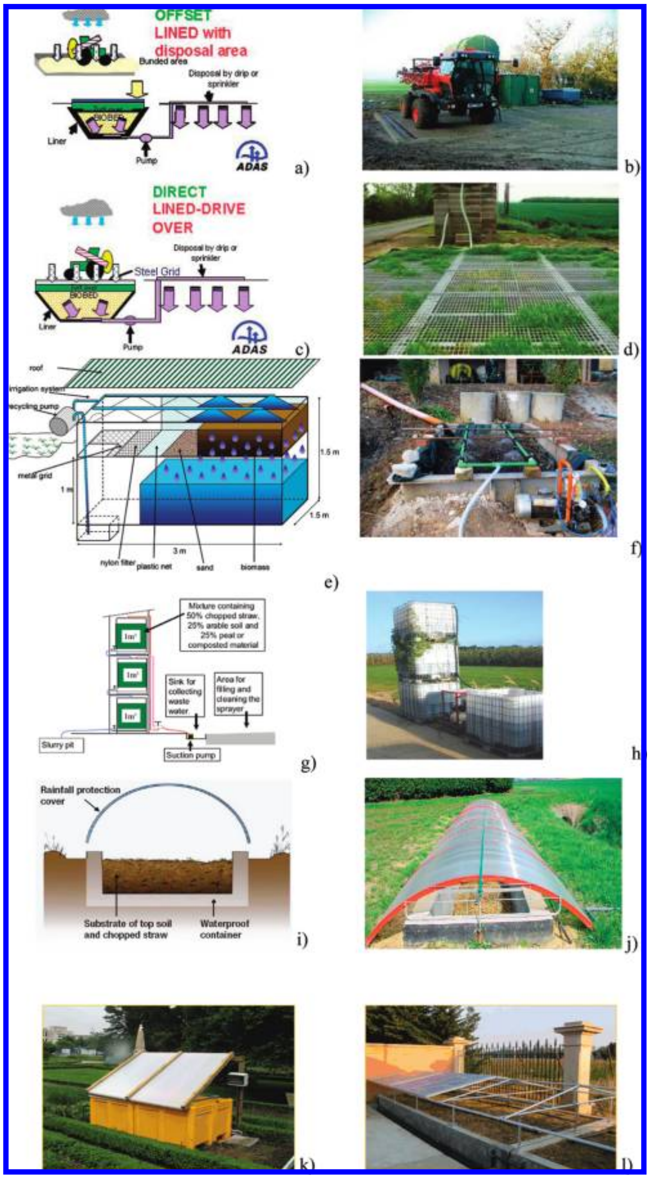

Figure 8. Bioprophylactic systems in other countries. Biobeds in the United Kingdom: (**a** and **b**) offset system and (**c** and **d**) direct system (http://www.biobeds.info). Biomassbed in Italy: (**e**) diagram and (**f**) pilot plant in a vineyard (*29, 30*). Biofilter in Belgium: (**g**) diagram (*31*) and (**h**) a field system of a three-unit biofilter with an extra unit (*32*). Phytobac in France: (**i**) diagram and (**j**) pilot system (*33*). Biobac system developed by INRA, France, for (**k**) small and (**l**) large effluent volumes (http://www.biotisa.com/english_version/english_explication3.htm).

Two systems were outlined, (i) an offset or indirect system where the handling area of the pesticides is separated from the biobed area (requires two collection tanks, one before and one after the biobed) (**Figure 8a**,**b**) and (ii) a drive-over system where the handling area is directly over the biobed area (requires a collection tank after the biobed). The liquids collected from the biobeds are drip-irrigated in designated disposal areas (**Figure 8c**,**d**). The biobed mixture in use in the United Kingdom consists of straw (wheat or barley), soil, and peat-free compost in the proportions 50:25:25 vol %, and the bed is covered with grass to ensure rooting activity and assist moisture management.

Studies in the United Kingdom regarding the effect of pesticide concentration, pesticide mixtures, repeated applications of pesticides, the effect of using different soils in the biomixture, and water management show that (i) pesticides degrade more slowly at higher concentrations, but this effect appears to be less significant in the biomixture than in soil alone (*34*); (ii) interactions between pesticides are possible but generally appear to be of smaller significance in the biobed than in soil alone (*36*); (iii) the biobed is able to cope with relatively complex mixtures of pesticides that are repeatedly applied (*36*); (iv) the use of different soil types in the biomixture has little impact on the overall efficiency of the biobed, so it is possible to use local soils (*24*); and (v) water management is crucial for the performance, cost of construction, and management of the biobed. With the exception of the most mobile pesticides (K_{oc} < 35), biobed performance is similar to that of more expensive treatment systems, with >99.9% of the applied pesticides retained and/or degraded within 12 months (*35*). The leaching potential in lined and unlined biobeds (*35*) was also studied and is discussed in section 4.2.

New exemptions to the agricultural waste regulations allow growers to install lined biobeds to treat pesticide washing water and runoff from pesticide handling areas. The new exemptions apply from May 2, 2007, in England and Wales, bringing them into line with Scotland, which has had a similar exemption since December, 2006. Growers must register their site with the Environment Agency (EA) before building a biobed, and they must follow the EA guidelines. The biobed must be situated at least 10 m away from water courses and 50 m away from a spring, well, or borehole. It can be used to treat up to 15000 L of dilute spray waste (excluding rainwater), with the drainage water being used to irrigate vegetated land. The biobed mix needs to be replaced every 5 years. Spent biobed mix can be spread on farmland after 12 months of storage (http://www.biobeds.info/content/default.asp).

3.2. Biobeds in Italy—Biomassbed. An Italian biobed system, still under development, is the biomassbed (**Figure 8e**,**f**), which utilizes biomixtures as filters through which pesticide-contaminated water is circulated and decontaminated (*29, 37, 38*) (www.biomassbed.it). The studies in Italy mainly focus on the treatment of large amounts of pesticide-contaminated water from the filling and washing of spraying equipment and on the use of local organic materials. Because peat is not easily found in Italy and is expensive, other organic materials are being tested as replacements (*37*). Materials such as urban and garden composts, peach stones, vine branches, and citrus peel have been tested because of their availability and cheapness (*38, 39*), and studies at laboratory scale using such mixtures have shown a high degradation of chlorpyrifos, metalaxyl, and imazamox (*38*). Also, 90% of pesticide contamination was removed from water treated in a prototype of the biomassbed installed in a vineyard and using a biomixture of vine branches, green compost, and topsoil (40:40:20 vol %) (*29*). Recently, the importance of straw in the Italian biomixture and the presence of an active lignin-degrading system have been demonstrated (*37, 40*). Although preliminary studies show efficient retention and degradation of the pesticides in the biomassbed, the long-term performance and final disposal of the exhausted biomixture have to be evaluated, especially with respect to the accumulation of copper in the biomass (*29, 39*).

3.3. Biobeds in Belgium—Biofilters. In Belgium, the biobed takes the form of a biofilter (**Figure 8g**,**h**), and laboratory and field-scale evaluations have been carried out on the degradation and leaching of pesticides, as well as the use of different organic materials in the biofilter (*9, 31*). The main interest in Belgium was to modify the biobed concept into a more flexible small system able to treat large volumes of effluents, to recycle them with a pump, and to use different kinds of substrates. Biofilters consist of two or three units of 1 m^3 plastic containers stacked in a vertical pile and connected with plastic valves and pipes. The choice between using a two-unit or a three-unit system depends on the sprayer (if it has a clean water reservoir), the amount of water to be treated, and the total pesticide load. A two-unit biofilter is recommended for loads in the range of 100 g of active ingredient and volumes of water of less than 3000 L per year. A three-unit system is recommended for higher loadings.

The biofilter substrate is a homogenized mixture of local soil, chopped straw and peat, or composted material or other materials (*31, 41*). For instance, composted farmyard manure has been used instead of straw and/or peat. Fresh material (such as straw and manure) is added each year to improve the capacity of the mixture (*31, 42*).

Among the drawbacks of the biofilter, probably because of the lack of a grass layer to balance the moisture, is that structural heterogeneities in the mixture can contribute to the formation of channels and increase the risk of preferential flow and consequently leaching of pesticides. In addition, maintenance is required to keep the correct moisture and aeration. Otherwise, carry-over from the first to the second year can occur with those pesticides that are not easily degraded, for example, lenacil (*31*). Plants are included in a later development of the biofilter (*32*). Biofilters are now registered by the Ministry of Agriculture and Environment of the Walloon Region in the southern part of Belgium and are recommended to pesticide users (*43*).

3.4. Biobeds in France—Phytobac and Biobac. Two biobed systems have been developed in France, Phytobac and biobac.

3.4.1. Phytobac. The Phytobac, developed by Bayer CropScience, was inspired by the Swedish concept of the biobed. It consists of a 60 cm deep basin made of watertight materials to ensure complete retention of contaminants and effluents (**Figure 8i**,**j**). The sides of the basin are 30 cm above soil level to avoid flooding from runoff. The substrate consists of topsoil from the farm (70%) and chopped straw (30%). No grass layer is placed on the top, and a cover protects the bed from rainfall. The Phytobac is intended to treat all of the contaminated volumes of water coming from tank waste and spillages during mixing/loading, rinsing, and cleaning of sprayers. It can be used alone or connected to the mixing/loading and sprayer cleaning area. In the latter case, it is connected to a buffer tank where the effluents are collected and recirculated onto the Phytobac. The water in the Phytobac is regulated by evaporation only, so moisture has to be managed to avoid saturation or drying of the materials (*33*).

The mixture of topsoil and straw readily degrades pesticides in less than 1 year, even at high concentrations. The conditions favorable to degradation in the Phytobac are similar to those in soils (temperature, humidity, etc.), and adaptation of microorganisms to pesticide degradation has been observed in the Phytobac as in soils. According to Guyot and Chenivesse (*33*), safe disposal of Phytobac contents in the field is possible (10 m^3 ha^{-1}). There is no evidence to indicate a need for completely replacing the Phytobac substrate, but fresh material has to be added at regular intervals (*44*).

The Phytobac generally comprises a large installation. Because of slow water evaporation from the water-tight system, large volumes of substrates are needed to avoid saturation or even overflowing (*31*). Furthermore, the upper layers tend to dry out and become hydrophobic, increasing the risk for fast water drainage. The Phytobac can also be difficult to protect from rainfall, clogging of pipe circuits can frequently occur, and it is difficult to mix the substrate and consequently to obtain a homogeneous mixture.

3.4.2. Biobac. The biobac (**Figure 8k**,**l**), developed by researchers at INRA, France, is another system derived from the Swedish biobed and the Phytobac. It consists of a tank insulated from the subsoil and filled with a mixture of organic and mineral materials, mainly soil from the farm and chopped straw. The concept behind this system is that farm soil contains microorganisms, which over successive treatments have adapted to the degradation of pesticides used at the farm, and this natural detoxifying ability of the soil microflora can be maintained and encouraged in the biobac by the input of a supplementary source of carbon and energy, such as straw. One of the differences in relation to the Phytobac system is that the moisture and aeration levels are controlled. In laboratory trials with 200 L biobac tanks, 100 g of pesticides was reduced to 0.6 g in 15 months. Currently, the biobac system is commercially available under the name Biobac from Biotisa (http://www.biotisa.com/english_version/english_explication1.htm), a company created in June 2005 by six scientists from INRA and CNRS, associated with specialists in agribusiness.

3.5. Biobeds in Denmark. Several studies have been performed in Denmark to adapt the original Swedish biobed design to Danish conditions. The main concern has been to guarantee that no pesticides leach from the biobed. Therefore, to assess potential leaching of pesticides, the lined biobed system with collection of effluents has been used as a model in pilot and field-scale studies. Laboratory trials also have been performed to study the retention and degradation of pesticides at different concentrations. An important difference with respect to the Swedish recommendations is that the biobed in Denmark is also intended to treat water from washing the spraying equipment.

Studies carried out under Danish meteorological conditions in a fully established full-scale model biobed (**Figure 9**) with a surface area of 15 m^2 showed that even with a turf layer on the top and a 10 cm clay membrane at the bottom, water from precipitation and cleaning of sprayers percolated through the biobed and reached the soil layers beneath. In the leachate, 11 of the 21 applied pesticides appeared at concentrations from 0.4 to 445 $\mu g\ L^{-1}$, while the remaining 10 were not detected. The most mobile compound in the study was bentazone, with 14% being recovered below the biobed, while the accumulated amounts of the other pesticides in the leachate were below 2% (*45*).

Overall, the pilot and field-scale studies showed that most of the applied amounts of the pesticides were retained and degraded in the biobed but that significant leaching of the most mobile types occurred. Those studies formed part of an evaluation by the Danish Environmental Protection Agency on Guidelines for Prevention of Pollution in Small Waterworks—Handling of Pesticides in Agriculture. On the basis of the results obtained, the authorities considered that the biobeds presented a potential risk for pesticide leaching and were not approved for use in Denmark. However, the biobed profile used in the studies may have promoted the leaching of pesticides. This topic is discussed in section 4.2.

3.6. Biobeds in Latin America. The inexpensiveness, simplicity, and efficiency of the biobed system make it suitable for application in developing countries. One important difference is the often smaller size of farms in developing countries as compared with European farms. Therefore, a small biobed for a person standing and filling a backpack sprayer is often sufficient.

The pilot biobed in Peru is located in the Carapongo valley, near Lima (**Figure 10a**). This area consists of small farms of less than 1 hectare that produce vegetables such as cabbage, lettuce, coriander, celery, beetroot, radish, and tomatoes. Straw and peat are not available in the area; therefore, the lignin-rich common reed (*Phragmites australis*), known locally as the weed carrizo, has been selected as the source of lignin as a substitute for straw, while garden compost is used as a substitute for peat. Local soils are used in the mixture. In laboratory trials, a mixture of 50:25:25 vol % of reed−compost−soil gave 40−80% mineralization of methomyl and methamidophos within 10 days of incubation at 30 °C (unpublished data). The purpose of the studies was to develop a validated biobed system that can be implemented in the area with the help of the health and agricultural authorities. Further studies are needed to evaluate the efficiency of biomixtures with other organic materials that can be used in different regions of the country.

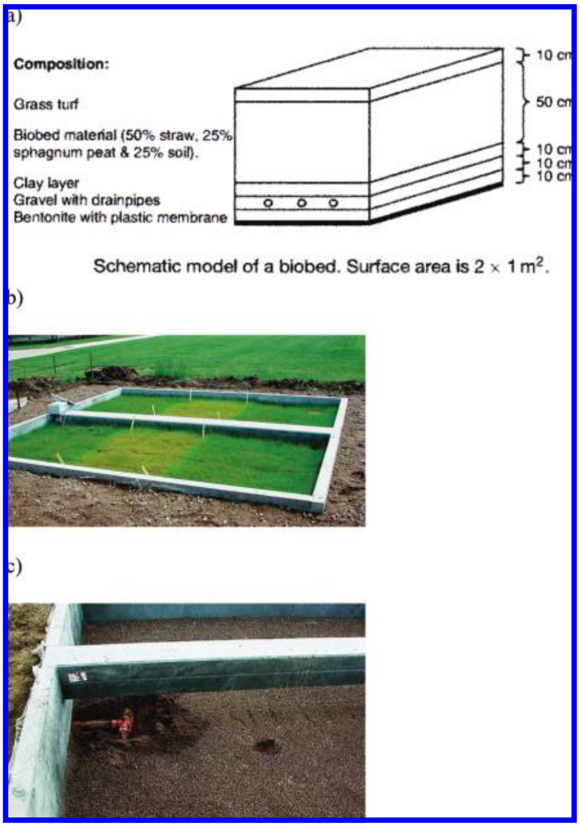

Figure 9. Studies of lined biobeds in Denmark. (**a**) Profile of the pilot biobed (2 m^2) (*25*). (**b**) The field-scale lined biobed (15 m^2) consisting of concrete elements. (**c**) Above the concrete floor is a 10 cm layer of gravel with a drainage tube, covered by a clay layer (*46*).

The biobeds in Guatemala are also designed for one person standing and filling a backpack sprayer (**Figure 10b**,**c**). However, in places where there is a risk of flooding, it is safer to use a biotable (**Figure 10d**,**e**). The biotable consists of a cylinder half-buried in the ground and containing all of the elements of a biobed [clay layer at the bottom, biomixture (maize residues, soil, and peat), and grass layer at the surface]. Because it is located above the ground level, it can be used as a table. The research on biobeds in Guatemala is carried out by Agrequima (http://www.agrequima.com.gt/) and the Universidad del Valle (http://www.uvg.edu.gt/), where, for instance, the effect of fungicides on the efficiency of biobeds is being evaluated at the laboratory scale.

In El Salvador, a program for the introduction of biobeds was initiated in 2006 by the Asociación de Proveedores Agrícolas (APA) as part of the project CropLife Latin America (http://65.36.216.53/croplife/CampoLimpio/RedAsociaciones.php).

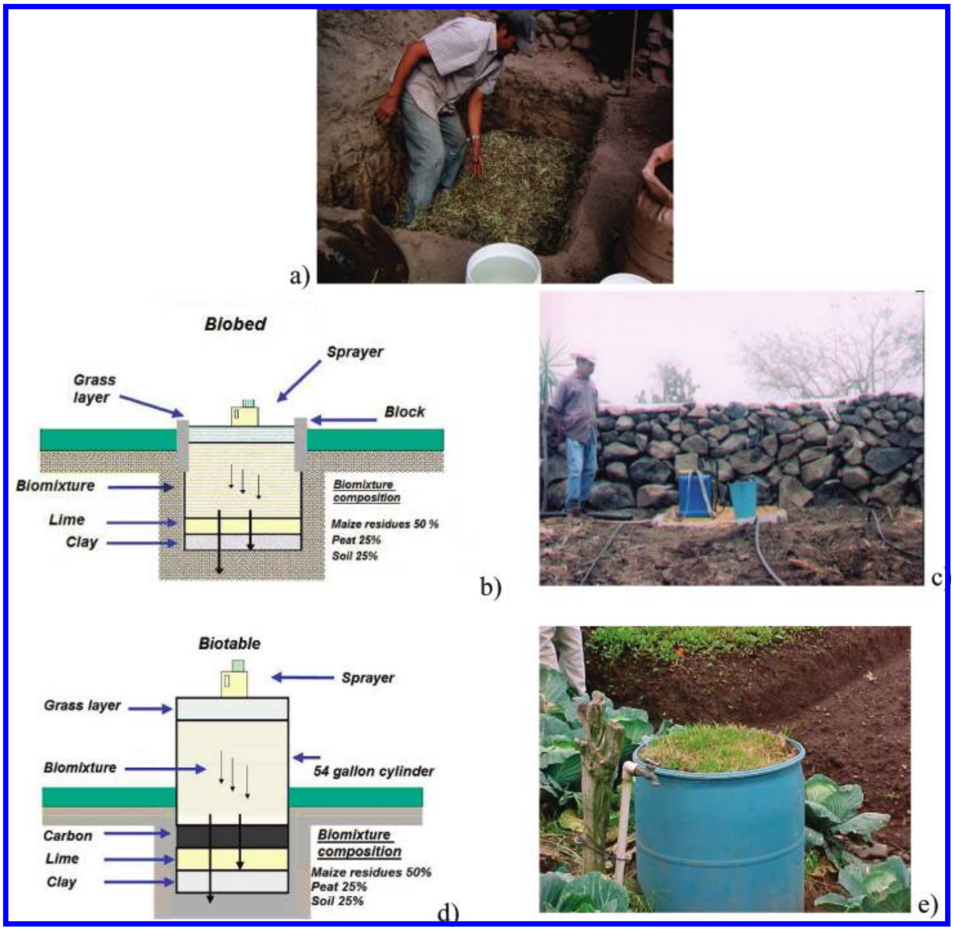

Figure 10. Biobeds in Latin America. (**a**) Construction of a biobed in the Carapongo valley, outside the city of Lima in Peru. Bioprophylactic systems in Guatemala: (**b**and **c**) a biobed and (**d** and **e**) a biotable. Pictures: Agrequima, www.agrequima.com.gt; the texts in the figures are translated to English by the authors.

A pilot biobed has been constructed in the department of Esmeraldas, north of Quito in Ecuador, by Agrofin Oil Palm & Tropical Fruit Producer, a company producing African palm oil. The biobed is part of a designated area for the management of pesticides. It includes storage space for the chemicals, storage space for contaminated materials, showers, and a biobed for the treatment of pesticide spills. The biobed is lined, and the effluents are collected and sprayed on active soil (Hugo Zumarraga, Agrofin Palm Oil, personal communication).

Recently, a new project has started in the province of Riobamba, where a community with more than 1500 small farmers is being educated on the safe management of pesticides (Hugo Zumarraga, Agrofin Palm Oil, personal communication). The work will include construction of a biobed for the sprayer/tractor and around 50 small biobeds of 1.5 m^2 each. The project is being coordinated by CESA, Central Ecuatoriana de Servicios Agrícolas, and the Universidad Andina Simón Bolívar. The funding comes from the International Fund for Agricultural Development (IFAD) and the Swiss Agency for Development and Cooperation (SDC).

Recently, a project has started in Mexico, at the Colegio de Postgraduados (COLPOS) in Montecillo, which will deal with biological processes for degradation of pesticides in biobeds with the purpose of adapting the system to Mexican conditions. Maize residues are planned to be used as the lignocellulosic material instead of straw (Katina Stamatiu, COLPOS, personal communication).

3.7. Biobeds in Other Countries. The technical potential of using biobeds to contain and degrade pesticides has been evaluated in a series of experiments using laboratory-scale biobeds located in greenhouses in Utah, United States. The study was performed by Earthfax Development Corporation and funded by the U.S. EPA. In general, the experiments involved application of selected herbicides to the surface of the biobeds, which were prepared to assess various factors (e.g., substrate mixtures with and without fungal inoculation). The herbicide-degrading potential of the biobed substrate mixtures was determined by analyzing soil/peat/straw (or corn stover or corn cob) mixture subsamples taken from various depths in the beds to determine residual herbicide concentrations over time. According to the results, the degradative performance of biobeds for several of the most commonly used herbicides in the United States was exceptional, particularly for the most heavily used herbicide in the United States, namely, atrazine (http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/1727/report/F). Studies at Lincoln University on biobeds were planned for the year 2001 (http://www.reeis.usda.gov/web/areera/POW.AES.1890.mo.plan.2001.wpd.pdf), but no results were available at the time of preparation of this review.

In The Netherlands, the biobed is designed to accommodate Dutch conditions, that is, it has to be built (partly) above the ground because in large parts of The Netherlands the watertable is very shallow. The size of the biobed is adapted to the expected volume of contaminated water. Pilot versions will be operated in 2008 (Wim Beltman, Wageningen University, personal communication).

Demonstration biobeds have been built in Estonia, Latvia, Lithuania, Poland, and Kaliningrad as part of the Improved

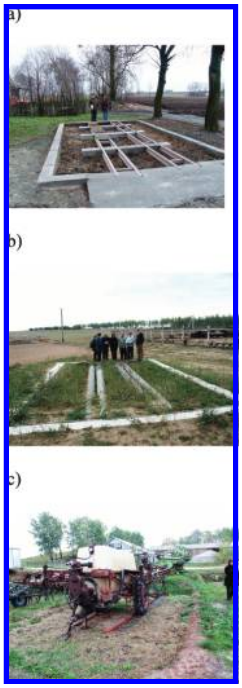

Figure 11. Demonstration biobeds in (**a**) Gutmanski, Poland and (**b**) Trakai and (**c**) Jonava in Lithuania. Pictures: Eskil Nilsson, VISAVI, Malmö, Sweden.

Pesticide Management in Agriculture project by Scanagri, Sweden (Eskil Nilsson, VISAVI, personal communication) (**Figure 11**). Evaluations of the biobed under Polish conditions have been performed by the Institute of Plant Protection (Poznan, Poland) (*47*). There are also some biobeds in Norway and Finland. Laboratory evaluations of biobeds have been performed in Vietnam, Uganda, India, and Sri Lanka (Kristina Mastroianni, Niras AB, personal communication), and projects with the Phytobac are being run in Morocco, Ivory Coast, Senegal, Portugal, and Benelux (*33*).

4. FACTORS AFFECTING BIOBED PERFORMANCE

The performance of a biobed is measured by its ability to retain and degrade pesticides. An efficient biobed involves all of its three components, that is, the clay layer, the biomixture, and the grass layer, working correctly. The degradation of pesticides is mainly determined by the properties of the biomixture. The grass layer contributes indirectly to the degradation by regulating the water balance for the biological processes in the biomixture and perhaps directly by phytoremediation processes. The retention of the pesticides mainly depends on the properties of the biomixture, the clay and the grass layers, and their interactions with the water in the biobed.

4.1. Effect of the Biomixture on Degradation and Sorption of Pesticides. A good biomixture promotes pesticide binding and an efficient and robust microbial flora with a durable pesticide degradation capacity able to tolerate pesticides at high concentrations, at repeated applications and in mixtures. Binding and degradation are affected by factors such as the (i) composition, (ii) homogeneity, (iii) age, (iv) temperature, and (v) moisture of the biomixture.

4.1.1. Effect of Biomixture Composition on Pesticide Degradation and Sorption. The composition of the biomixture is crucial for pesticide sorption and the type of microbial activity prevailing, that is, the amount, activity, and genotypic and phenotypic versatility of the microorganisms responsible for the degradation of pesticides and their metabolites, as well as the robustness of the system as regards high concentrations, mixtures, and repeated applications of pesticides. The OSB consists of straw, peat, and soil. The introduction of other bioprophylactic systems often results in modifications of this original recipe as regards components and their proportions. Here, we discuss (i) the effect on degradation and binding of each component of the OSB and of other components and compositions that are used in other biomixtures, (ii) the effect of composition on the microbial activity, and (iii) the effect of composition on the robustness of the biomixture.

Figure 12. White rot fungi in the OSB.

4.1.1.1. Role of the Biomixture Components in Pesticide Degradation and Binding. It seems to be generally accepted that straw or some other lignin-rich component should be present in the biomixture of the various systems developed today (*11, 14, 33, 37, 41, 48*). Depending on its availability in the specific country/region, the straw in the biomixture is sometimes replaced by other materials, for example, citrus peel, vine branches, chitin, coconut byproduct, and composted farmyard manure (*32, 38, 42, 49*). Additional information about other materials used in the biomixtures can be found in De Wilde et al. (*9*).

Organic materials with a small or no lignin content or a high nitrogen level may not support sufficient microbial activity for the degradation of pesticides and their metabolites. In a recent study, Coppola et al. (*37*) showed that the use of mixtures of urban compost and citrus peel led to accumulation of 3,5,6-trichloropyridinol (TCP), a chlorpyrifos metabolite, while mixtures containing increasing levels of straw from vine branches gave decreasing levels of TCP (*37*). Furthermore, despite higher respiration rates obtained, the degradation of chlorpyrifos by citrus peel was less effective than that by straw. Thus, high respiration rates do not always give a high pesticide degradation rate, probably because qualitatively appropriate microbial activity is also required. Therefore, if straw is not available, other lignocellulosic materials should be considered. For instance, lignin-rich reeds have been used in Peru and maize residues in Guatemala instead of straw due to its limited availability in the study areas. However, data are not yet available on the precise microbial processes prevailing in the presence of these materials.

In general, the use of lignocellulosic materials has several practical advantages: They are often available on farms, they are persistent, and their slow degradation allows a continuous supply of carbon, energy, and nutrients without the need for frequent additions. However, the level of the lignocellulosic material in the biomixture has an effect on the type of microbial activity that develops (see section 4.1.1.2).

The peat in the biomixture contributes to sorption capacity, pH regulation, and moisture control and also abiotic degradation processes, as observed for terbuthylazine in laboratory biobeds (*14*). This has been corroborated by the results of Fournier (*50*), who observed that the absence of peat in the biomixtures of the Phytobac/biobac gives a slow degradation of terbuthylazine. Peat may also have an effect on the activity of the phenoloxidases by causing their complexation with humic acids and thereby keeping them in the soil solution, albeit with reduced activity due to the presence of tannins, polyuronic acids, and phenols, which can act as phenoloxidase inhibitors (*15, 51*). The peat is also important in regulating the pH of the OSB to levels suitable for phenoloxidase activities (*14*). By replacing peat with other materials, such regulation can be altered and too high of a pH for phenoloxidase activity can be attained.

In many countries, peat can be expensive, not easily available, or considered as a nonsustainable resource (*9, 37*). Peat-free compost is considered more environmentally friendly and is therefore used in the United Kingdom (*35, 36, 52, 53*), while garden and urban composts have been used in Italy (*37, 38*). Despite lower respiration rates, garden compost was found to be more efficient than urban composts in degrading chlorpyrifos and its metabolite TCP. De Vleeschouwer et al. (*54*) compared peat and green waste compost and found that degradation with the green waste compost was less efficient. The pH of the biomixtures containing green waste compost was 7.5−8.0, which is probably too high to promote efficient lignin-degrading fungi. Composted cow manure has also been used instead of peat and gave higher efficiency in the biomixture of biofilters (*43*). However, no data are available on the pH values of such biomixtures and on the microbial activities prevailing under those conditions. Further studies are also needed to evaluate the effect of replacing peat in the biomixture and the subsequent change in pH on the efficiency of pesticide retention. For instance, a high pH may increase the mobility of certain pesticides (e.g., some sulfonylurea pesticides).

The soil provides sorption capacity and also microorganisms other than ligninolytic fungi capable of degrading pesticides. The soils found on farms are normally used in preparation of the biomixtures. These soils may have acquired special abilities to degrade the pesticides that are frequently used on the farms. This ability is mainly exploited in the Phytobac/biobac biomixtures, where the bacterial soil activity is promoted by low amounts of straw and a neutral pH (*33, 48*).

The effect of different soil types on leaching and degradation of pesticides in laboratory-scale biobeds was studied by Fogg and Boxall (*24*). They found that there were no significant differences in the biobed performance and, therefore, that local soils can be used in the biomixture. However, the use of soils with high clay content, besides giving practical handling problems, could decrease the availability of slowly degrading pesticides with time (aging). Further research is needed to study such aging processes in the biomixture.

4.1.1.2. Effect of Biomixture Composition on Microbial Activity. The biomixture composition determines the microbial activities that prevail. Few studies have been carried out to correlate pesticide degradation with microbial activity in the biobeds or similar systems. The only available information comes from the studies with the Swedish biobeds and the Phytobac and biobac in France, and these data are restricted to the presence of straw in the biomixture. The studies in Sweden have focused on using a biomixture that can promote the fungal degradation of pesticides with the help of white rot fungi, while with the Phytobac and biobac in France, the purpose is to promote the bacterial degradation of pesticides, mainly by bacteria originating from the soil.

Cometabolic degradation of pesticide mixtures by the lignin-degrading system of white rot fungi provides the main microbial activity in the OSB, with its lignin-rich straw (Figure 12) and low pH of about 5.9 due to the presence of peat (*14*). White rot fungi are important lignin degraders, and it has been shown that the lignin-degrading extracellular ligninolytic enzymes (phenoloxidases) are responsible for the degradation of a broad range of organic pollutants (*55–60*) including pesticides (*15, 16, 18, 19, 61*) by nonspecific free radical mechanisms. The phenoloxidases include peroxidases (for example, manganese and lignin peroxidases) and polyphenoloxidases (for example, laccases).

Strong positive correlations between straw content, respiration rate, and phenoloxidase activities have been observed in laboratory-scale biobeds (*14*). Moreover, most pesticides (metamitron, metribuzin, methabenzthiazuron, isoproturon, and linuron) were found to be dissipated by cometabolic processes, and their dissipation was correlated to the levels of straw, respiration, and/or phenoloxidase activities. Degradation kinetics typical for metabolic processes were observed in the case of chloridazon only but were preceded by a long phase of cometabolic degradation (approximately 80 days at 20 °C) where chloridazon dissipation was correlated to straw and phenoloxidase activity. The low nitrogen levels in the biomixtures, designed to favor activation of lignin-degrading enzymes, may limit metabolic processes. The lignin-degrading system of many white rot fungi is nitrogen-regulated (*62*); at low nitrogen levels, the fungi activate the production of phenoloxidases, while higher levels can enhance growth but inhibit the production of the enzymes. Therefore, the addition of nitrogen to the biomixtures is not recommended in Sweden. No information is available on the type of microbial activity in other systems, but principal component statistical analyses have shown that high levels of nitrogen (NH_4) also decrease the efficiency of biofilters in Belgium (*43*).

It can be argued that as compared with metabolic degradation processes, cometabolic processes, as in the degradation of pesticides under ligninolytic conditions, can be slower and that the high levels of straw used can temporarily decrease the bioavailability of the pesticides by sorption. However, such concerns are not crucial as long as the pesticides are retained in the biobed and the degradation occurs within 1 year. However, special attention should be given to pesticides that require longer degradation periods and that can have a tendency to accumulate.

Metabolic degradation of pesticides by bacteria from the soil is the activity prevailing in the Phytobac/biobac systems. The biomixture used in these cases consists of 70 vol % of soil and 30 vol % of straw and has a pH of 7, which favors bacterial activity and especially bacteria from the farm soil, which over successive treatments have adapted to degradation of the pesticides used on the farm (*63*). The conditions favorable to degradation in Phytobac/biobac are the same as those in soils (temperature, moisture, etc.), and a capacity for adaptation of microorganisms has been observed in the Phytobac/biobac, as in soils. The potential of diuron, atrazine, and isoproturon mineralization strongly increased in soils that were pretreated (7 months previously) with the respective pesticides. Moreover, the addition of straw to an adapted soil did not affect the mineralization of the pesticides, as shown for atrazine (*50*). The addition of cow manure (5%) or natural composts (30%) 7 months before application of bentazon, atrazine, and isoproturon increased their mineralization rate in adapted soils. Metabolic processes such as those prevailing in the Phytobac/biobac systems may require the addition of nitrogen to support the growth and/or activity of the microorganisms.

Metabolic degradation is an interesting process because it can transform the pesticide into nontoxic products such as biomass,

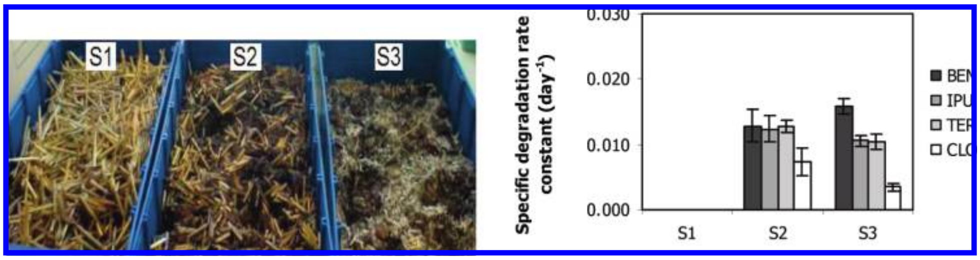

Figure 13. Effect of straw size on the degradation of pesticides. (**a**) Three straw lengths were used in the preparation of the biomixtures: 5 (S1), 2 (S2), and <0.2 cm (S3). (**b**) Specific degradation rate constant (d^{-1}) of bentazon (BEN), isoproturon (IPU), terbuthylazine (TER), and chlorpyrifos (CLO) in the different biomixtures.

CO_2, and water. However, some concerns may arise regarding microorganisms with an enhanced ability to degrade pesticides and how they should be discarded in the environment, since enhanced pesticide degradation can reduce the efficacy of the pesticide. Moreover, there is no information about the robustness of such processes in relation to the use of mixtures of pesticides at high concentrations and at repeated applications.

4.1.1.3. Biomixture Composition and Robust Microbial Activity. An important property of a biobed mixture is that it promotes a robust microbial flora with an efficient and durable pesticide degradation capacity able to tolerate pesticides at high concentrations, at repeated applications, in mixtures and interactions among them.

Interactions can occur when mixtures of pesticides are degraded, such as competition for degrading enzymes (*14*) or inhibition of the degradation of certain pesticides due to the formation of toxic metabolites from the degradation of other pesticides present. For example, TCP, a chlopryrifos metabolite, has antimicrobial activity and can inhibit further degradation of chlorpyrifos and other processes (*37*).

Pesticide degradation rates can decrease with increasing concentrations of pesticides (*25, 34*). Moreover, Fogg et al. (*34, 36*) observed that the degradation of pesticides applied to the biomixture as a mixture was slower than when the compounds were applied individually. Furthermore, repeated applications of some pesticides gave the same effect, that is, decreasing degradation rates with each additional treatment. However, all of the effects were less significant in the OSB than in soil only.

In contrast, Fournier (*50*) observed that repeated applications of pesticides produced enhanced degradation processes in the soils used in biobac systems. The ability persisted for at least 7 months and was not affected even by additions of straw. These contradictory results are explained by De Wilde (*9*) as a concentration factor, that is, the pesticide concentrations used by Fogg et al. (*36*) were significantly higher than those used by Fournier (*50*). Another explanation could be due to the existence of two different microbial systems, a cometabolic, mainly fungal, system occurring in the OSB that is not enhanced by repeated applications of pesticides and a metabolic system, mainly bacterial, occurring in the biobac biomixture, the rate of which increases with enrichment of degrading microorganisms.

The robustness of the OSB has been demonstrated in a recent study in which it was compared with an Italian biomixture as regards the ability to degrade chlorpyrifos (*40*). The Italian biomixture consisted of straw (vine branches), urban/garden compost, and Italian soil in proportions of 40:40:20 vol %. The results showed, for example, that (i) the half-life of chlorpyrifos in the Swedish biomixture was not different from that in the Italian biomixture, despite a higher initial chlorpyrifos concentration, and (ii) the respiration activity in the Swedish biomixture was not negatively affected by the presence of chlorpyrifos as it was in the Italian biomixture, despite the higher chlorpyrifos concentration used in the Swedish biomixture. The authors concluded that it is important to include in the biomixture lignin-rich materials that can favor the lignin-degrading activity for pesticide degradation.

Even though there are no published studies about microbial activity and degradation of pesticides in the other bioprophylactic systems, it can be assumed that as long as the OSB is used, the lignin-degrading system will provide a good pesticide degradation activity. However, it is unclear how this activity is affected when the peat in the biomixture is replaced with composted cow manure or compost material (*43*), since the increase in nitrogen in the biomixture and the increased pH may affect the activation of the lignin-degrading enzymes. Therefore, further studies are needed to evaluate the microbiology of the biomixtures containing composted cow manure or compost material instead of peat. Studies of the microbiology of systems in which the straw has been replaced with other lignocellulosic materials are also required.

4.1.2. Biomixture Homogeneity and Effect of Straw Length. Good mixing of the biomixture components is important to give (i) a homogeneous substrate to support the growth and activity of the pesticide-degrading microorganisms and (ii) a good sorption capacity without preferential flow paths. The homogeneity of the biomixture depends on different factors such as the efficiency of the mixing process, for example, by use of a blender. However, a more important factor is the length of the straw. The use of long straw, for example, unchopped straw, gives (i) a heterogeneous biomixture, with the formation of pockets of different microbial and sorption capacities; (ii) a decreased specific area in the biomixture, which may give slower degradation rates and inadequate sorption capacity; and (iii) a risk of preferential flow paths. In addition, longer straw gives lower levels of straw in the biomixture as the volumetric weight is lower than for straw of shorter size.

Recently, we studied the effect of straw length on the degradation of pesticides in laboratory trials (unpublished data). Three straw lengths, 5, 2, and <0.2 cm, were used in the preparation of OSBs. The heterogeneity of the biomixture with longer straw size as compared with the shorter sizes was visually evident (**Figure 13**). Higher specific degradation rates were also observed in the OSBs with shorter straw size, whereas no significant degradation was observed in the biomixture with longer straw length during the study period (93 days). The preparation and pretreatment, for example, by precomposting, of the biomixture thus is important to ensure the presence of an efficient pesticide degradation capacity already when the biomixture initially is added to the biobed.

4.1.3. Biomixture Age. The progressive degradation of the organic material generates a succession of microbial activities and substrates being degraded, which could affect the function of the biobed by (i) changing the degradation capacity, leading to slower pesticide degradation and metabolite accumulation, which in turn could give toxic effects on the microbial amount and activity, and (ii) by affecting the sorption capacity, which in turn could increase the mobility of pesticides in the biobed.

The sorption capacity in young biobeds can be critical. Studies on lined biobeds in Denmark (*25*) showed that a newly built biobed with a fresh biomixture and poor grass and clay layers (see section 4.2) subjected to high water loadings (worst-case scenario) can leach pesticides. However, the leaching was minimized after 1 year even when the clay layer was probably still nonfunctional (see section 4.2). The risk of pesticide leaching from young biobeds with functional clay layers is probably small, but the poor sorption capacity of the young biomixture may allow transport of the chemicals to the bottom of the biobed (on top of the clay layer), where they may be slowly degraded due to oxygen limitation.

Studies with biofilters have shown carry-over of some pesticides from the first to the second year (*31*). In laboratory-scale biobeds, leaching of pesticides occurred after the first application in a young biomixture, while the pesticide retention became more efficient after the second (5 months later) and third applications (1 year later). These studies also showed that the retention of pesticides was better when reused soil and peat were used to prepare the biomixture. It was therefore concluded that an aged biomixture should be present in the system to increase retention capacity. However, care must be taken when using recycled material from the biobeds, since persistent pesticides can still be present in the exhausted biomixture and their mobility could be enhanced during the handling of the material. Further studies are necessary.

Despite annual additions of a new layer (10 cm) of fresh biomixture on the top of the biobed (to compensate for the degradation of the organic material and the consequent decrease in the volume of the biomixture), the carbon content in the core of the biomixture decreases with time (unpublished data). Lower carbon contents give lower respiration rates and phenoloxidase activities, which can lead to decreased pesticide degradation efficiency (*14*). However, young biomixtures give inefficient pesticide retention, and a certain maturity is therefore needed to improve retention. A good practice can be to precompost the biomixture before adding it to the biobed. This practice has already been introduced in the United Kingdom. Further studies are needed to examine the effect of age of the biomixture on biobed efficiency.

4.1.4. Biomixture Temperature. As in all biological processes, the degradation of pesticides is affected by the temperature in the biobeds. In laboratory trials, 20 °C gave higher dissipation rates than 2 and 10 °C for all of the pesticides studied except for chloridazon (*14*). Increasing temperatures increase microbial and enzymatic activities, for example, phenoloxidase activity (*14*), but can also increase the solubility of the pesticides. No effect of temperature was seen on the dissipation rate of chloridazon. Other factors could have been more important in this case, for example, the development of chloridazon degraders, which could have been restricted by the low nitrogen content in the biobed mixture (*14*). Low temperatures in the Swedish climate probably often limit activity in Swedish biobeds, with most of the pesticide degradation occurring during the spring and summer, with smaller but still significant rates in the autumn (*14*).

Temperature also has an effect on the degradation of the organic material. While complete replacement of the exhausted biomixture is recommended every 5−8 years in Sweden, more frequent replacement is probably needed in warmer climates. This frequency must be determined specifically in each country/region.

4.1.5. Biomixture Moisture Content. Ideally, the moisture in the biobed should be high enough to promote microbial processes and solubilization of pesticides but still leave enough pore space for oxygen to support aerobic processes. Moreover, moisture levels near saturation increase the risk of transport of chemicals from the biobed and promote anaerobic processes (*64*). The effect of three moisture levels (30, 60, and 90% of water holding capacity, WHC) has been studied in laboratory biobeds (*14*). Moisture at 60% of WHC gave the highest dissipation of most of the pesticides tested, while moisture at 30 and 90% of WHC limited the microbial activity. However, regulating the moisture content in farm biobeds may be a difficult task; therefore, it is important to include peat or similar water-binding materials in the biomixture.

4.2. Biobed Water Management. Oversaturation with water can occur in the biobed, for example, when the sprayer is washed on the biobed (*65, 66*). To avoid this situation, Swedish biobeds should not be used for washing of the sprayer. Instead, an extra water container for washing the equipment in the field is recommended. Persistent rainfall can also cause oversaturation of biobeds, and in such cases, covering of the biobeds is recommended (*25*). In Sweden, it is also recommended that biobeds in areas with high precipitation should be covered from late autumn and during the winter period.

A problem observed in biobeds and the Phytobac is that the absence of peat in the biomixture, and also a nonfunctioning grass layer, can give a hydrophobic crust at the top of the biomixture (*25, 26, 35, 45*). Such crust formation can reduce microbial activity and promote transport of pesticides to the bottom of the biobed, where the clay layer thus has an important role in preventing leaching and increasing the time for degradation processes. However, to be efficient, the clay layer must not develop cracks that can give preferential flow paths for pesticide leaching.

According to Swedish studies of unlined biobeds, most of the pesticides are retained in the upper 20 cm of the biobed, with concentrations below the limit of detection in the clay layer at the bottom, suggesting limited downward transport (*10*). However, there have been some studies in Denmark and the United Kingdom reporting leaching of pesticides from lined biobeds.

In the studies carried out in Denmark, model (*25*) and field (*45*) biobeds were used. The model biobed (2 m^2) had a profile (**Figure 9**) consisting of a plastic membrane at the bottom, a layer of bentonite (10 cm), a 10 cm layer of gravel with drainpipes conducting the percolate to a reservoir, a 10 cm layer of clay, and 50 cm of a typical biomixture (straw−peat−soil) at the top. Isoproturon and MCPP were added to the biobed (8 g of each pesticide) together with 80 mm of water. The field biobed consisted of a concrete pit with an area of 15 m^2. The bottom was filled with gravel (10 cm) and a drainage tube leading to a collection well for sampling of the percolate. The gravel was covered by a 10 cm layer of rammed clay. The remaining volume was filled with a typical biomixture. This field biobed received 21 pesticides (5 g of each) and a total of 40 mm of water. Isoproturon (mean concentration, 0.22 mg L^{-1}) and MCPP (mean concentration, 2.09 mg L^{-1}) were detected in the percolate of the model biobed. In the field biobed, 10 of the 21 pesticides were found in the percolate, with bentazone showing the highest concentration (mean concentration, 0.17 mg L^{-1}).

In the UK study, two sets of lysimeters were prepared using PVC piping (19 cm internal diameter × 75 cm length) with one end of the pipe sealed using a socket fitted with a drain outlet (*35*). Cores were filled with 2−3 cm of gravel followed by 15 cm of washed sand. A 50 cm layer of biomixture was packed into each lysimeter. The biomixture was prepared by mixing topsoil, peat-free compost, and unchopped winter barley straw in the proportions 25:25:50 vol %. The results showed that only the most mobile pesticides leached, and for these, >99% was retained by the system, with a significant proportion degraded within 9 months. Peak concentrations of 127 and 50 $\mu g\ L^{-1}$ for the two most mobile pesticides, isoproturon and dimethoate, respectively, did however exceed the limits that are likely to be required by regulatory bodies.

There are several factors that can have interacted to give such high pesticide leaching from these biobeds. For good function of a biobed, all of the parts of the biobed must be working properly. Furthermore, for pesticides to leach from a biobed, they have to go through three layers, the grass, the biomixture, and the clay layer.

In the English study, the biomixture was used as a model of the biobed, but no grass or clay layers were included in the system, while even though the grass and the clay layers were present in the Danish studies, they may have functioned improperly. The absence of a well-established grass layer, as in the case of the Danish study, does not allow efficient water removal by root uptake and evapotranspiration and also promotes the formation of a dry, hydrophobic crust on top of the biomixture (see section 2.3.3).

The clay layer was absent in the English study, and in the Danish study, it was placed above a drainage layer. This could have promoted pesticide leaching by preferential flow as a consequence of the formation of cracks in the clay by drying, due to broken capillary forces in the drainage layer below the clay and exposure to the atmosphere through the drainage pipes. Furthermore, in the lined biobeds used in the Danish study, capillary water transport was eliminated by the impermeable layer at the bottom, and the use of unchopped straw in the English study may have contributed to the leaching from the lysimeters.

Both the Danish and the English reports propose that a deeper biobed (100−150 cm instead of 50 cm) may retain the pesticides for a longer period, giving time for more pesticide degradation before they reach the bottom of the biobed. However, deeper biobeds may cause accumulation of pesticides in the lower parts due to limited degradation by oxygen and limitations on biological activity. An alternative could be to increase the efficiency of the clay layer at the bottom by ensuring that it is kept moist, by increasing its thickness, or by using another efficient sorbent. Further studies are needed.

Another factor that may have an effect on the mobility of pesticides in a biobed or similar systems is the time between the pesticide spill and the rainfall and/or washing events. It has been shown that the time from application to the time when drain flow begins is the dominant factor in determining concentrations and losses in drain flow of a moderately mobile and persistent herbicide such as isoproturon (*67*). That study showed that the decrease in concentrations and total losses of isoproturon in drain flow with increasing time from application to the first drainage flow was significantly greater than would be expected from degradation alone. The availability for leaching probably decreases with time due to increased sorption (aging) (*67*).

Such aging could also contribute to explain the leaching of pesticides in the Danish and English studies, where the water loadings were made at the moment of pesticide application (Danish study) or shortly afterward (English study). It might therefore be necessary to cover the biobeds if heavy rainfalls events are expected shortly after pesticide spills.

5. CONCLUSIONS AND RESEARCH NEEDS

Biobeds originated in Sweden as a response to the need for simple and effective systems to minimize environmental contamination from pesticide use, especially when filling the spraying equipment, a typical point source of contamination. Water management is a delicate issue in biobeds and similar systems. In Sweden, biobeds are intended exclusively for the treatment of pesticide spills when filling and storing the spraying equipment. Rainwater is allowed into the biobed, but the sprayer has to be washed in the field. The biobed should be considered as a unit consisting of three components, the clay, the biomixture, and the grass layers, and each of these components has an important role for retention and degradation of pesticides. The first biobeds in Sweden were built in 1993, and it is estimated that more than 1500 biobeds are now in use in the country.

The biobed system has attracted attention in a number of other countries, which have initiated work to adapt it to their particular conditions. As a consequence, the biobed system has been modified and sometimes renamed, for example, as biomassbed in Italy, biofilter in Belgium, Phytobac and biobac in France, and biotable in Guatemala. When the biobed is used in retention and degradation of pesticides originating from sprayer washing, the construction has to be adapted to, for example, include a lining layer to ensure that no pesticide leaching can occur. The replacement of some of the original materials in the biomixture can also change the performance of the system, for instance, the amount, activity, and composition of the microbial community that develops.

The effectiveness and simplicity of biobed systems make them suitable for use in developing countries. Biobeds and similar systems are now spread all over the world, and it is important to analyze their actual performance in situ and to identify research needs.

5.1. Research Needs. More studies are needed to evaluate the effect of changing the straw to other lignocellulosic materials and of replacing peat by compost in the biomixture, since the subsequent change in pH may increase the mobility of certain pesticides and favor processes other than the efficient and nonspecific fungal activities. Better practices for achieving a more homogeneous biomixture in terms of mixing and of smaller particle size must be investigated to enhance degradation and retention of pesticides. More research is needed to assess the changes in pesticide degradation and binding efficiency that occur during maturation of the biomixture. A freshly made biomixture can give an inefficient pesticide-binding capacity, and the progressive degradation of the organic material eventually decreases the pesticide-degrading capacity of the biomixture. Therefore, a good practice can be to precompost the biomixture before adding it to the biobed and to completely replace it when the microbial activity becomes too low (after 6−8 years in Sweden). Further research is needed to evaluate the role of the grass layer on the degradation of pesticides by phytoremediation processes. More “active” grass types can probably be identified. High temperature has an effect on microbial activity, on degradation of the organic material, and on solubility of the pesticides. The implications of this are faster degradation, more frequent addition of fresh organic material, and higher mobility of pesticides. Therefore, studies of the efficiency of biobeds in warmer countries than Sweden are needed. Moisture is important for microbial activity and for the solubilization of the pesticides. Low moisture levels limit microbial activity, while high levels (near saturation) may limit aerobic processes by oxygen deficiency. Simple systems are required to regulate and measure the moisture in field biobeds. Leaching of pesticides has been observed from lined biobeds. However, this might be an artifact of the biobed profile used. The presence of a drainage layer under the clay layer may have allowed desiccation and crack formation in the clay barrier and the consequent leaching of pesticides from the biobed by preferential flow. Unlined biobeds with an added clay layer may also carry a risk of pesticide leaching if the clay is placed above a natural drainage layer. Further studies are needed to minimize this effect. The retention time of the pesticides in the biobed can be increased by increasing the depth of the biobed or by using a more effective adsorptive layer at the bottom of the bed. However, an increased depth might cause accumulation of the most mobile pesticides at the bottom of the biobed, where anaerobic conditions may develop. Further studies are needed to evaluate alternatives to the conventional clay layer for a more efficient impermeable layer at the bottom of the biobed, for instance, a thicker clay layer or use of other sorbents.

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LITERATURE CITED

(1) Helweg, A. Threats to water quality from pesticides—Case histories from Denmark. *Pestic. Outlook* **1994**, *5*, 12–18.

(2) Spliid, N. H.; Brüsch, W.; Jacobsen, O. S.; Hansen, S. U. Pesticide point sources and dispersion of pesticides from a site previously used for handling of pesticides. 16th Danish Plant Protection Conference, Side Effects of Pesticides. *Weeds* **1999**, 33−46.

(3) Stenvang, L.; Helweg, A. Minimizing pollution risk at filling and washing sites for sprayers. 17th Danish Plant Protection Conference, Overview/Environment/Weeds, **2000**, 73−77.

(4) Fischer, P.; Hartmann, H.; Bach, M.; Burhenne, J.; Frede, H. G.; Spiteller, M. Gewasserbelastung durch pflanzenschutzmittel in drei einzugsgebieten. *Gesunde Pflanz.* **1998**, *50*, 142–147.

(5) Fischer, P.; Hartmann, H.; Bach, M.; Burhenne, J.; Frede, H. G.; Spiteller, M. Reduktion des gewasserreintrags von pflanzensschutzmitteln aus punktquellen durch beratung. *Gesunde Pflanz.* **1998**, *50*, 148–152.

(6) Frede, H. G.; Fischer, P.; Bach, M. Reduction of herbicide contamination in flowing waters. *Z. Pflanzenernahrung Bodenkunde* **1998**, *161*, 395–400.

(7) Kreuger, J. Pesticides in the environment—Atmospheric deposition and transport to surface waters. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 1999.

(8) Torstensson, L. Biobäddar skyddar miljön. *Fakta Mark/Växter 4*; Swedish University of Agricultural Sciences: Uppsala, Sweden, 1995.

(9) De Wilde, T.; Spanoghe, P.; Debaer, C.; Ryckeboer, J.; Springael, D.; Jaeken, P. Overview of on-farm bioremediation systems to reduce the occurrence of point source contamination. *Pest Manage. Sci.* **2007**, *63*, 111–128.

(10) Torstensson, L. Experiences of biobeds in practical use in Sweden. *Pestic. Outlook* **2000**, *11*, 206–212.

(11) Torstensson, L.; Castillo, M. d. P. Use of biobeds in Sweden to minimize environmental spillages from agricultural spraying equipment. *Pestic. Outlook* **1997**, *8*, 24–27.

(12) Torstensson, L.; Börjesson, E. Elvärmd biobädd skyddar grundvatten vid växthuset. *Fakta Trädgård 1*; Swedish University of Agricultural Sciences: Uppsala, Sweden, 2002.

(13) Castillo, M. d. P.; Torstensson, L. Biobeds—Biotechnology for environmental protection from pesticide pollution. In *Methods and Techniques for Cleaning-up Contaminated Sites*; Annable, M. D., Teodorescu, M., Hlavinek, P., Diels, L., Eds.; Springer: Berlin, 2008.

(14) Castillo, M. d. P.; Torstensson, L. Effect of biobed composition, moisture and temperature on the degradation of pesticides. *J. Agric. Food Chem.* **2007**, *55*, 5725–5733.

(15) Castillo, M. d. P.; Ander, P.; Stenström, J. Lignin and manganese peroxidase activity in extracts from straw solid substrate fermentations. *Biotechnol. Tech.* **1997**, *11*, 701–706.

(16) Castillo, M. d. P.; Ander, P.; Stenström, J.; Torstensson, L. Degradation of the herbicide bentazon as related to enzyme production by *Phanerochaete chrysosporium* in a solid substrate fermentation system. *World J. Microbiol. Biotechnol.* **2000**, *16*, 289–295.

(17) Castillo, M. d. P.; Andersson, A.; Ander, P.; Stenström, J.; Torstensson, L. Establishment of the white rot fungus *Phanerochaete chrysoporium* on unsterile straw of solid substrate fermentation systems intended for degradation of pesticides. *World J. Microbiol. Biotechnol.* **2001**, *17*, 627–633.

(18) Castillo, M. d. P.; von Wirén-Lehr, S.; Scheunert, I.; Torstensson, L. Degradation of isoproturon by the white rot fungus *Phanerochaete chrysosporium*. *Biol. Fertil. Soils* **2001**, *33*, 521–528.

(19) von Wirén-Lehr, S.; Castillo, M. d. P.; Torstensson, L.; Scheunert, I. Degradation of isoproturon in biobeds. *Biol. Fertil. Soils* **2001**, *33*, 535–540.

(20) Torstensson, L. Herbicides in the Environment. *Proceedings of the Second International Weed Control Congress*, Copenhagen, Denmark, 25−28 June 1996; Vol. 1−4, 267−274.

(21) Bergström, L.; Stenström, J. Environmental fate of chemicals in soil. *Ambio* **1998**, *27*, 16–23.

(22) Kotterman, M. J. J.; Vis, E. H.; Field, J. A. Successive mineralization and detoxification of benzo[a]pyrene by the white rot fungus *Bjerkandera* sp. strain BOS55 and indigenous microflora. *Appl. Environ. Microbiol.* **1998**, *64*, 2853–2858.

(23) Vaughan, D.; Cheshire, M. V.; Ord, B. G. Exudation of peroxidase from roots of *Festuca rubra* and its effects on exuded phenolic acids. *Plant Soil* **1994**, *160*, 153–155.

(24) Fogg, P.; Boxall, A. B. A. Effect of different soil textures on leaching potential and degradation of pesticides in biobeds. *J. Agric. Food Chem.* **2004**, *52*, 5643–5652.

(25) Henriksen, V. V.; Helweg, A.; Spliid, N. H.; Felding, G.; Stenvang, L. Capacity of model biobeds to retain and degrade mecoprop and isoproturon. *Pest Manage. Sci.* **2003**, *59*, 1076–1082.

(26) Spanoghe, P.; Maes, A.; Steurbaut, W. Limitation of point source pesticide pollution: Results of bioremediation system. *Commun. Agric. Appl. Biol. Sci.* **2004**, *69*, 719–732.

(27) Torstensson, L.; Olsson, G.; Norup, S.; Stenberg, B. Biobäddar Minskar Miljörisker vid Fyllning av Lantbrukssprutor; 35th Swedish Crop Protection Conference; Swedish University of Agricultural Sciences: Uppsala, Sweden, 1994; pp 223−233.

(28) Kreuger, J.; Nilsson, E. Catchment scale risk-mitigation experiences—Key issues for reducing pesticide transport to surface waters. Presented at BCPC Symposium Proceedings No. 78: Pesticide Behaviour in Soil and Water, 2001; pp 319−324.

(29) Fait, G.; Nicelli, M.; Fragoulis, G.; Trevisan, M.; Capri, E. Reduction of point contamination sources of pesticide from a vineyard farm. *Environ. Sci. Technol.* **2007**, *41*, 3302–3308.

(30) Fait, G.; Nicelli, M.; Trevisan, M.; Capri, E. Un sistema biologico per decontaminare da agrofarmaci acque di provenienza aziendale. *Informatore Agrario* **2004**, *60*, 43–45.

(31) Pussemier, L.; Vleeschouwe, C. d.; Debongnie, P. Self-made biofilters for on-farm clean-up of pesticides wastes. *Outlooks Pest Manage.* **2004**, *15*, 60–63.
(32) Debaer, C.; Jaeken, P. Modified biofilters to clean up leftovers from spray loading and cleaning; experience from pilot installations. *Aspects Appl. Biol.* **2006**, *77*, 247–252.
(33) Guyot, C.; Chenivesse, D. A simple and affordable system to prevent water contamination. ICM EDITION, Bayer CropScience, September 2006, *2006*, 31−33.
(34) Fogg, P.; Boxall, A. B. A.; Walker, A. Degradation of pesticides in biobeds: The effect of concentration and pesticide mixtures. *J. Agric. Food Chem.* **2003**, *51*, 5344–5349.
(35) Fogg, P.; Boxall, A. B. A.; Walker, A.; Jukes, A. Degradation and leaching potential of pesticides in biobed systems. *Pest Manage. Sci.* **2004**, *60*, 645–654.
(36) Fogg, P.; Boxall, A. B. A.; Walker, A.; Jukes, A. A. Pesticide degradation in a "biobed" composting substrate. *Pest Manage. Sci.* **2003**, *59*, 527–537.
(37) Coppola, L.; Castillo, M. d. P.; Monaci, E.; Vischetti, C. Adaptation of the biobed composition for chlorpyrifos degradation to southern Europe conditions. *J. Agric. Food Chem.* **2007**, *55*, 396–401.
(38) Vischetti, C.; Capri, E.; Trevisan, M.; Casucci, C.; Perucci, P. Biomassbed: A biological system to reduce pesticide point contamination at farm level. *Chemosphere* **2004**, *55*, 823–828.
(39) Trevisan, M.; Fait, G.; Nicelli, M.; Capri, E. In A Biological System To Reduce the Point Contamination of Surface Waters, Proceedings of the 3rd European Conference on Pesticides and Related Organic Micropollutants in the Environment, Halkidiki, Greece, 7–10 October, 2004; Albanis, T. A., Ed.; Halkidiki, Greece, 2004; pp 177–178.
(40) Vischetti, C.; Coppola, L.; Monaci, E.; Cardinali, A.; Castillo, M. d. P. Microbial impact of the pesticide chlorpyrifos in Swedish and Italian biobeds. *Agron. Sustainable Dev.* **2007**, *27*, 267–272.
(41) Pigeon, O.; Vleeschouwer, C. d.; Cors, F.; Weickmans, B.; Ryckel, B. d.; Pussemier, L.; Debongnie, P.; Culot, M. Development of biofilters to treat the pesticides wastes from spraying applications. *Commun. Agric. Appl. Biol. Sci.* **2005**, *70*, 1003–1012.
(42) Genot, P.; Huynh, N. v.; Debongnie, P.; Pussemier, L. Effects of addition of straw, chitin and manure to new or recycled biofilters on their pesticides retention and degradation properties. *Mededelingen−Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen* **2002**, *67*, 117−128.
(43) Pigeon, O.; De Vleesschouwer, C.; Cors, F.; Huyghebaert, B. P. V.; Pussemier, L.; Culot, M. *Biofilters To Treat the Pesticides Wastes from Spraying Applications: Results after 4 Years of Study*; Wallon Agricultural Research Centre (CRA-W), Pesticides Research Department: Gembloux, 2006.
(44) Pietrantoni, B. Promoting development of biobeds/Phytobacs by Bayer CropScience in France. *European Biobed Workshop*; Bayer CropScience: Malmö, Sweden, 2004.
(45) Spliid, N. H.; Helweg, A.; Heinrichson, K. Leaching and degradation of 21 pesticides in a full-scale model biobed. *Chemosphere* **2006**, *65*, 2223–2232.
(46) Spliid, N. H. Biobed research in Denmark. In *European Biobed Workshop*; Husby, J., Ed.; Bayer CropScience: Malmö, Sweden, 2004.
(47) Gorecka, H.; Wolny, S. A "biobed" a filling and cleaning stand for sprayers for Polish farms. *Ochrona Roslin* **2003**, *47*, 19–20.
(48) Fournier, J. C.Bioremediation applications to decompose pesticide wastes in farm: A necessity of knowledge on the microbial behaviour and the environmental risk. InterCOST Workshop on Bioremediation, Sorrento, Italy, Sorrento, Italy, 2000; pp 89−92.
(49) Jaeken, P.; Debaer, C. On farm processing of pesticide contaminated water in fruit production. In *European Biobed Workshop*; Husby, J., Ed.; Bayer CropScience: Malmö, Sweden, 2004.
(50) Fournier, J.-C. A survey of INRA studies on biobeds. In *European Biobed Workshop*; Husby, J., Ed.; Bayer CropScience: Malmö, Sweden, 2004.
(51) Wetzel, R. G. Extracellular enzymatic interactions: Storage, redistribution and interspecific communications. In *Microbial Enzymes in Aquatic Environments*; Cróst, R. J., Ed.; Springer-Verlag: New York, United States, 1991; pp 6−28.
(52) Fogg, P. Biobeds: Safe disposal of pesticide waste and washings. Presented at BCPC Symposium No. 78: Pesticide Behaviour in Soils and Water, Brighton, United Kingdom, 2001; pp 217−223.
(53) Fogg, P.; Boxall, A. B. A.; Walker, A.; Jukes, A. Leaching of pesticides from biobeds: Effect of biobed depth and water loading. *J. Agric. Food Chem.* **2004**, *52*, 6217–6227.
(54) De Vleeschouwer, C.; Pigeon, O.; Cors, F.; De Ryckel, B.; Weickmans, B.; Meeus, P. *Developpement de Bio-Epurateurs Destines a Traiter Les Eaux De Rincage et de Nettoyage des Pulverisateurs*; Centre Wallon de Recherches Agronomiques, Departement Phytopharmacie: Gembloux, Belgium, 2005.
(55) Aust, S. D. Degradation of environmental pollutants by *Phanerochaete chrysosporium*. *Microb. Ecol.* **1990**, *20*, 197–209.
(56) Aust, S. D.; Benson, J. T. The fungus among us−Use of white rot fungi to biodegrade environmental pollutants. *Environ. Health Perspect.* **1993**, *101*, 232–233.
(57) Bumpus, J. A. Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **1989**, *55*, 154–158.
(58) Bumpus, J. A.; Aust, S. D. Biodegradation of DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] by the white rot fungus *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **1987**, *53*, 2001–2008.
(59) Field, J. A.; de Jong, E.; Feijoo Costa, G.; de Bont, J. A. M. Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics. *Trends Biotechnol.* **1993**, *11*, 44–49.
(60) Kennedy, D. W.; Aust, S. D.; Bumpus, J. A. Comparative biodegradation of alkyl halide insecticides by the white rot fungus *Phanerochaete chrysosporium* (BKM-F-1767). *Appl. Environ. Microbiol.* **1990**, *56*, 2347–2353.
(61) Castillo, M. d. P. Degradation of pesticides by *Phanerochaete chrysosporium* in solid substrate fermentation. Doctoral Thesis, Swedish University of Agricultural Sciences, 1997.
(62) Waldrop, M. P.; Zak, D. R. Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. *Ecosystems* **2006**, *9*, 921–933.
(63) Fournier, J.-C.; Martin-Laurent, F.; Catroux, G. Detoxifying plant health product effluents from farms. http://www.international.inra.fr/press/detoxifying_plant_health.
(64) Fomsgaard, I. S. Degradation of pesticides in subsurface soils, unsaturated zone−A review of methods and results. *Int. J. Environ. Anal. Chem.* **1995**, *58*, 231–245.
(65) Basford, W. D.; Rose, S. C.; Carter, A. D. On-farm bioremediation (biobed) systems to limit point source pesticide pollution from sprayer mixing and washdown areas. *Aspects Appl. Biol.* **2004**, *71*, 27–34.
(66) Spliid, N. H.; Helweg, A. Fate of Pesticides in a Full Scale Model Biobed. Presented at the International Symposium Non-Agricultural Use of Pesticides−Environmental Issues and Alternatives, Copenhagen, Denmark, 2003; pp 57−58.
(67) Jones, R. L.; Arnold, D. J. S.; Harris, G. L.; Bailey, S. W.; Pepper, T. J.; Mason, D. J.; Brown, C. D.; Leeds-Harrison, P. B.; Walker, A.; Bromilow, R. H.; Brockie, D.; Nicholls, P. H.; Craven, A. C. C.; Lythgo, C. M. Processes affecting movement of pesticides to drainage in cracking clay soils. *Pestic. Outlook* **2000**, *11*, 174–177.

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