



A review on bioremediation of food industry wastes

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Abstract

Bioremediation is management of waste products such as biological and chemical wastes which involves use of various techniques. Generally bioremediation is carried out through naturally occurring organisms that can neutralize the contaminants. Composting and wastewater treatments are recognizable examples of old environmental biotechnologies so environmental biotechnology is not a new field. Bio treatment is well accepted by industry as it goes along with the current popularity of maintaining nature's harmony. Bioremediation has become a widely accepted option for the cleanup of contaminated soils and aquifers although it does not have a fully credible reputation within the regulatory community. Food industries produces liquid waste with many common characteristics, such as high BOD and COD, but differ in the concentration of the organic compounds that causes major problems in land and water resources.

Keywords: bioremediation, composting, food industry, waste bioremediation

Introduction

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site."

According to the EPA, Bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances."

A few decades ago, man's greatest challenge resided in speeding up the industrialization process. Today man attempts to find ways to deal with the growing industrialization and the associated problems. A third of Europe's 300 million hectares of dry lands suffer from desertification and the ensuing reduction in biological and economic productivity (UNEP, 1992). These effects have brought in the idea of Bioremediation. Bioremediation is the naturally occurring process by which microorganisms either stop or renovate environmental contaminants to inoffensive end products. Bioremediation is rising as a promising technology for the treatment of soil and groundwater contamination.

Bioremediation is an important soil and groundwater remediation strategy because it:

- Harnesses naturally occurring bio geological processes;
- Destroys or immobilizes contaminants rather than transfers them from one environmental medium to another; and
- Conserves financial resources due to shortened cleanup times and/or lower capital expenditures to many other remediation technologies (GZA Geo Environmental, 1998).

Principle of bioremediation

Composting and wastewater treatments are recognizable examples of old environmental biotechnologies so environmental biotechnology is not a new field. However, recent studies in ecology and molecular biology suggest

opportunities for more competent biological procedures. Clean-up of polluted water and land areas are notable accomplishments of these studies. Bioremediation is defined as under controlled conditions, the process whereby organic wastes are biologically degraded to an innocuous state, or to levels underneath meditation limits established by regulatory establishment. When the import of microorganisms take place in a contaminated site to enhance the degradation that we have a process known as bio augmentation (Vidali, 2001).

Bioremediation has its limitations like other technologies. Some contaminants like high aromatic hydrocarbons or chlorinated organic, are defiant to microbial attack. They deteriorate slowly or not at all, so it is not easy to predict the rates of the cleaning exercise in the biological treatment, there are no rules to predict whether it can be contaminated with degraded. Biological treatment techniques are usually more economical than conventional methods such as incineration and can treat some of the contaminants at the site, thus reducing the risk of personal hygiene or exposure to the widest possible result of traffic accidents. (Colberg and Young, 1995).

Methods of Bioremediation

Bio treatment is well accepted by industry as it goes along with the current popularity of maintaining nature's harmony. Bioremediation has become a widely accepted option for the cleanup of contaminated soils and aquifers although it does not have a fully credible reputation within the regulatory community (NRC, 1993). There are numerous examples of employing bioremediation against various pollutants. Nowadays, there are four main biological techniques for treating soil and groundwater:

- a. Stimulation of the activity of indigenous microorganisms by the addition of nutrients, regulation of redox conditions, optimizing pH conditions, etc;

- b. Inoculation of the site by microorganisms with specific bio transforming abilities;
- c. Application of immobilized enzymes; and
- d. Use of plants (phyto remediation) to remove and/or transform pollutants (Bollag & Bollag, 1995)^[5]. In the specific methods used for bio remediating contaminated soil and water, land farming, composting, intrinsic bioremediation and slurry bioreactor are included (Table 2).

Land farming was most probably introduced into the scientific literature by an article describing disposal by biodegradation of oily sludges in soil (Dibble & Bartha, 1979). From an engineering perspective, landfarming is a “managed treatment and ultimate disposal process that involves the controlled application of a waste to a soil or soil-vegetation system” (Loehr, Asce, & Overcash, 1985)^[17].

Land farming relies on the principles applied in agriculture and aims at controlling the bio cycling of natural compounds. The biodegradation conditions by the natural indigenous microbial populations of soil are optimized by the dilution of contaminated soil with clean soil, tilling of the soil to reduce initial toxicity, as well as by controlling physical parameters, such as aeration, pH, soil moisture content, and temperature.

Composting is a biological aerobic decomposition of organic materials in which conditions are strictly controlled in order to help the thermophilic microorganisms to transform organic materials into a stable, soil like product (Miller, 1993; Rynk, 1992). The composting process is initiated by mesophilic bacteria, which are biologically active at temperatures between 30 and 45°C. Degradation of the organic matter results in heat production through exothermic reactions. Therefore, the temperature increases to 50–60°C thus facilitating the growth of thermophilic bacteria. The thermophilic bacteria may further increase the temperature with their activity and, if the conditions are not carefully controlled, the temperature may exceed 70°C, thus leading to lower activity. In order to avoid this and achieve maximum efficiency, conditions need to be optimized. This means optimizing oxygen concentration, pH, moisture content, carbon to nitrogen (C: N) ratio and particle size (Miller, 1993; Rynk, 1992). Within that frame, bulking agents such as wood chips and vermiculite have been successfully used to increase the void space in the compost (Baker, 1994)^[4]. During composting, the volume of material undergoes a substantial decrease in the order of 25–40% according to some researchers (Willow, 1992), while according to others it may even exceed 50% (O’Leary, Walsh, & Razvi, 1989–1990).

In slurry bioreactor treatment systems, the contaminated soils are excavated and mixed with water to form a slurry that is mechanically aerated in a reactor vessel. The reactor contents are agitated to promote breakdown of soil aggregates, enhance desorption of contaminants from soil solids, increase contact between the wastes and microorganisms, and enhance oxygenation of the slurry (Baker, 1994)^[4]. Different substances, such as surfactants, dispersants and materials supporting microbial growth, are added to the slurry to improve the treatment of contaminated soil and increase the biodegradation capability (United States Environmental Protection Agency, 1990). Temperature is also controlled to minimize microbial

growth. The concentration of the biomass is equally important for the maintenance of the degradation so microorganisms may be added to the slurry both in the beginning and during the process. King, Long, and Sheldon (1992) mentioned that in many cases the contaminated soils are pretreated before they are introduced into the reactor. The physical grading of soil reduces the cost of mixing and agitation. Fractionation of soils may reduce the total volume which needs to be treated and increase the rate of biodegradation of the contaminants (Portier, 1989). Other researchers have suggested that additional treatment may be necessary, such as addition of sodium hydroxide and sodium chloride to neutralize soil acidity and dispersion of clay particles to trap the contaminants (Black, Ahlert, Kosson, & Brugger, 1991; Baker, 1994)^[4].

Slurry bioreactors generally have a higher cost than the in-situ systems because of the high degree of engineering involved. Still the biodegradation rates of the same compound are faster in slurry bioreactors compared to the ones obtained by the in-situ technique (Castaldi & Ford, 1992; Stroo, 1989).

Bioremediation is divided into two broad categories: in-situ and ex-situ.

In-situ bioremediation: In-situ bioremediation is a natural process taking place ever since the first microbes and excess organic substance were both present in the soil (Litchfield, 1993)^[14]. In this process there is no need to excavate or remove soils or water in order to accomplish remediation. This method exploits natural ways of recycling nutrients through the cycles of nitrogen and carbon. These cycles nowadays are utilized by man to augment the degradation and recycling of wastes and the similar cycles are employed by in-situ bioremediation to hygienic contaminated soils (Nelson *et al.*, 1996). In this process, the disintegration of the contaminants is carried out by the indigenous microorganisms which grow on this contaminated soil and can only endure in that environment by using the contaminating substances as a source of energy (Aelion *et al.*, 1987; Litchfield, 1993)^[2, 15]. In in situ bioremediation, organic pollutants are completely distressed; therefore no secondary waste stream is produced (Dott *et al.*, 1995).

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These microorganisms have either been forced by the environmental conditions to adapt or die or have been genetically modified (Ellis & Gorder, 1997). Should one wish the microbial decomposition to continue, more nutrients, strictly selected after screening, should be added to the soil (Litchfield, 1993) ^[15]. The nutrient addition is performed through drills in the soil. In some cases and when there is no adequate control in the nutrient distribution it is not certain whether the substances reached their target or whether other regions have been also attained by the nutrients. This of course implies that the remediation process will be prolonged and the ecology of another area has been disturbed (Litchfield, 1993; Ogunseitan, Tedford, Pacia, Sirotkin, & Sayler, 1987) ^[14]. Generally, in-situ bioremediation is more difficult to keep under control than ex-situ or engineered bioremediation because experimental controls are usually unavailable in contaminated soils (Wilson & Jawson, 1995).

Ex situ bioremediation: Ex situ bioremediation techniques can be faster, easier to control and used to treat a wider range of contaminants and soil types than in situ techniques. This process requires excavation of contaminated soil or pumping of groundwater to facilitate microbial degradation. One of the main advantages of ex-situ bioremediation is that it requires less time than the in-situ treatment. Common ex situ treatments include land farming, windrows and bio piling. Bioremediation of organic compounds has been successfully employed at many sites, however physical factors can be rate limiting. Bio piling enhances aerobic catabolism of creosote by inoculation of air into piles of contaminated soil.

A complete ideal biotransformation system would include (Burton, 2001):

- An efficient enzyme production system
- using a readily culturable microbial source
- genetically stable, non-pathogenic strain
- An efficient biocatalyst
- used as resting cells or purified enzymes
- highly stereoselective
- high activity in the desired reaction
- flexible substrate selectivity
- minimal side reactions
- Stable biocatalyst
- Stable under optimal reaction conditions amenable to immobilisation/stabilisation (van Beilen and Li, 2002).

Polyphenoloxidases

Polyphenoloxidases (PPO) are oxidoreductases that catalyse oxidation of phenolic compounds (Durán and Esposito, 1997). They are subdivided into two subclasses, laccases and tyrosinases, and both groups react with oxygen and no cofactors are needed (Steffens *et al.*, 1998; Chevalier *et al.*, 1999).

Laccases

Laccases (EC 1.10.3.2, *p*-diphenol: dioxygen oxidoreductase) was first described by Yoshida (1883) and was characterised as a metal containing oxidase by Bertrand (1985). They are cuproproteins that catalyse the oxidation of several aromatic and inorganic substances (phenols) with the concomitant reduction of oxygen to water (Xu, 1996). In general, laccases contain four neighbour copper atoms, which are distributed among different binding sites and are classified into three types: copper type 1, 2 and 3 which are

differentiated by specific characteristic properties that allow them to play an important role in the catalytic mechanism of the enzyme (McMillin and Eggleston, 1997; McGuirl and Dooley, 1999). Laccases are characterised by low substrate specificity and their catalytic competence varies widely depending on the source. Simple diphenols are good substrate for the majority of laccases, but guaiacol and 2, 6-dimethoxyphenol are generally better substrates (Gianfreda and Bollag, 2004; Yaporolov *et al.*, 1994) ^[5]. Laccase is also able to catalyse the oxidation of other substituted polyphenols, aromatic amines, benzothioles and a series of other compounds but the enzyme, unlike tyrosinases, is unable to oxidase tyrosine (Thurston, 1994). Laccase is widely distributed in higher plants (Mayer and Harel, 1979), in fungi (Karam and Nicell, 1997) and in some bacterial strains of *Azospirillum lipoferum* (Givaudan *et al.*, 1993) and *Alteromonas* sp. (Sanchez-Amat and Solano, 1997).

Tyrosinases

Spectroscopic studies of tyrosinase (EC 1.14.18.1, monophenol:monooxygenase) have shown that its coupled binuclear copper active site is very similar to that found in hemocyanins (Sanchez-Ferrer *et al.*, 1995). It's well known that tyrosinases catalyses two different oxygen-dependent reactions that occur consequently: the *o*-hydroxylation of monophenols to yield *o*-diphenols (cresolate activity) with molecular oxygen in the presence of a chemical reductor and the subsequent oxidation of *o*-diphenols with molecular oxygen to *o*-quinone (catecholase activity). Quinones are usually formed rapidly, and undergo non-enzymatic conversion to form more stable intermediates that subsequently undergo slow oligomerisation reactions ultimately yielding high molecular weight, insoluble polyphenolics (Dec and Bollag, 1995; Naidja *et al.*, 1998) ^[5]. Typical substrates for tyrosinase besides phenols are also *p*-hydroxy- and 3, 4-dihydroxyphenylpropionic acids (Kahn *et al.*, 1999) and caffeic acid (Rompel *et al.*, 1999). This kind of enzyme is widely distributed throughout the phylogenetic scale from bacteria to mammals and even present different characteristics in different organs of the same organisms, such as in roots and leaves of higher plants (Burton, 1994).

Enzyme Immobilisation

An effective use of enzymes may be hampered by some peculiar properties of the enzymatic proteins such as their non-reusability, high sensitivity to several denaturing agents and presence of adverse sensory or toxicological effects. Many of these undesirable constraints may be removed by the use of immobilised enzymes (Chaplin and Bucke, 1990). In addition, immobilisation or stabilisation of the biological agent can also permit the containment of cells or the support of growing biofilm for use in a continuous system (bioreactors).

Besides, toxicity of immobilisation reagents should be considered in connection with the immobilisation process, waste disposal and final application of the immobilised enzyme catalyst (Messing, 1975). Many methods have been applied for laccase and tyrosinase immobilisation on solid supports, generally involving chemical or physical mechanisms (Zaborsky, 1974).

- Chemical immobilisation methods mainly include:

- enzyme attachment to the matrix by covalent bonds
- cross-linking between enzyme and matrix
- Enzyme cross-linking by multifunctional reagents.
- Physical immobilisation methods involve:
 - entrapment of enzyme within a porous hollow fibre or spun fibres
 - entrapment of enzyme within an insoluble gel matrix and/or a reverse micelle
- Adsorption of enzyme on different carriers.
- Both techniques offer advantages and disadvantages.

In general, chemical immobilisation offers stable enzyme attachment reducing enzyme deactivation rates and usefully altering its specificity. At the same time the enzyme activity could be reduced, for the presence of covalent bonds changing enzyme native structure. Entrapment and adsorption are milder techniques for enzyme perturbation. To this end, a very exhaustive review on immobilised laccase and tyrosinase applications has been recently proposed by Durán *et al.* (2002).

Bioremediation in Fermentation Industry

The fermentation industry is divided into three main categories: brewing, distilling and wine manufacture. Each of these industries produces liquid waste with many common characteristics, such as high BOD and COD, but differ in the concentration of the organic compounds that determine the biological treatment that will be selected. The difficulty in dealing with fermentation wastewaters is in the flows and loads of the waste, depending on the raw materials used.

Wine Industry

Molasses are sugar-production by-products with a high carbohydrate content (around 50%) that are extensively used as a cheap carbon source in many important industrial fermentation processes. The effluent generated during ethanol production from fermentation of sugar-cane molasses are called vinasses.

This final effluent produces an important ecological impact due to its high content of soluble organic matter and its intense dark-brown colour (mainly melanoidins). In fact, vinasses represent a major environmental problem for the ethanol-production industry and they are considered as the most aggressive by-product generated by sugar-cane factories. Most of the organic matter present in the vinasses can be diminished by conventional anaerobic-aerobic digestion, but the colour is hardly removed by these treatments (Valdez and Obaya, 1985) making this effluent a potential water pollutant blocking out light from rivers and streams thereby preventing oxygenation by photosynthesis and provoking their eutrophication. The enzymatic removal of phenolic compounds from must and wine has been reported on the laboratory scale using immobilised laccase from a mutant strain of *T. versicolour* (Brenna and Bianchi, 1994) while *Trametes* sp. I-62, was tested in bioremediation of distillery wastewaters (Gonzalez *et al.*, 2000). In this study, maximum effluent decolourisation values and COD reduction attained after 7 days of fungal treatment were 73.3 and 61.7%, when 20% (v/v) vinasses were added to the culture medium. Under these conditions, a 35-fold increase in laccase production by *Trametes* sp. I-62 was measured, but no manganese peroxidase activity could be detected. The higher increase in laccase activity observed also

corresponded with a higher effluent decolourisation value, suggesting that laccase overproduction may play the key role in effluent decolourisation. Gonzalez Benito *et al.* (1997) conducted laboratory batch tests to examine the ability of *Trametes versicolour* to treat molasses-based distillery waste waters. All the conditions affecting the treatment of waste, such as pH, nutrients and carbon source, were tested at various concentrations to determine their relation to the reduction of COD, decolourisation and decrease of ammonium content in the wastewaters. Satisfactory results were obtained working with a low sucrose concentration and adding KH₂PO₄ as the only nutrient. In this way, 82% colour elimination, 77% COD removal and 36% ammonium decrease were attained. PPO was isolated from *Coriolus* ssp. liquid cultures and immobilised on polyvinyl alcohol fibres. The immobilised derivative was successfully used for purification of wastewater from hydrolysis yeast industry. In fact, the treatment reduced the lignohumic acid content and colour index of the effluent by over 70 and 81–82%, respectively, and the immobilised biocatalyst retained its activity after 15 consecutive cycles (Gusarova *et al.*, 1989).

Bioremediation in Olive Oil Industry

Olive mill wastewaters (OMW) are a characteristic by-product of olive oil production and one of the major environmental problems in the Mediterranean area. It is estimated that during November and February each year, about 30 million m³/year of wastewater is generated. The liquid waste, a dark coloured juice, contains water (83–92%), minerals (1–2%) and organic substances (4–16%), such as sugars, organic acids, polyalcohols, colloids, tannins and lipids. The difficulty of disposing olive oil mill wastewater is mainly related to its high BOD, COD and concentration of organic substances, in particular phenols (from 1.5 to 8.0 g/L) which make aerobic degradation a difficult and expensive task (Saez *et al.*, 1992). High phenol and organic acid concentrations in OMW were shown to increase phytotoxicity under certain conditions, thus rendering biodegradability very difficult and the final compost non-usable. Two different approaches have been conducted on this effluent: sewage disposal and spreading on soil. The first procedure requires an overall COD reduction below legal limits, while this is not necessary for sparging which is much less expensive and allows complete exploitation of both the fertilising and irrigation potential of the OMW. However, as already stated, a drastic reduction in phenol concentration is a prerequisite for both techniques. The removal of polyphenols from waste has been extensively studied. Martirani *et al.* (1996) reported a decreasing content in phenolic amount of the OMW effluent using purified laccase from *Pleurotus ostreatus*, but no reduction of its toxicity was observed, Flouri *et al.* (1996) also used species of the same fungi to decolourise the OMW which gave positive results in 17–30 days. More recently, Tsioulpas *et al.* (2002) investigated in laboratory cultures the ability of several *Pleurotus* spp. Strains to remove phenolic compounds from OMW, with respect to their laccase activity.

The performance and enzymatic strategy exhibited by basidiomycete Euc-1, a laccase producing strain, was investigated during the biodegradation of OMW (Dias *et al.*, 2004). This strain removed 90% of phenols (initial concentration 800mg/L), 73% of colour and 45% of

chemical oxygen demand in batch cultures containing OMW. Since partial phenol removal occurred before the detection of enzymatic activity, no plausible correlation could be established between them. In contrast, decolourisation occurred only after the detection of laccase activity and coincided with its production over time, and several lines of evidence have strongly indicated that colour removal is clearly a laccase dependent extracellular process. In a recent study Quarantino *et al.* (2003) tested the reduction of OMW phenols content using the white-rot fungus *Panus tigrinus*. The OMW was employed either as such, diluted with tap water 1:2 or added with different nitrogen sources. Batch fermentations were carried out with effluent either as such, after partial recycle of the waste fermented with *Panus tigrinus* or after enzymatic pre-treatments. With a partial recycle of the fermented waste or after enzyme treatment, the fermentation time was reduced and good phenol content decrease was obtained. Greco *et al.* (1999) compared the utilisation of a polyphenoloxidase naturally immobilised in olive husk and purified laccase from *Trametes versicolour* for bioremediation of this effluent. Both enzymatic systems showed relevant activity towards phenol polymerisation, although olive husk seemed much more appealing because of its availability and the extremely low cost associated with excellent enzymatic activity and specificity. OMW biodegradation has been also investigated by white-rot fungi immobilisation (Vassilev *et al.*, 1997; D'Annibale *et al.*, 1998). In the first study, OMW supplemented, or not supplemented, with ammonium sulphate and rock phosphate was applied as the medium in a shake-flask repeated-batch fermentation with *Aspergillus Niger* immobilised on polyurethane sponge. The results showed higher growth of the immobilised mycelium and significant reduction of the total phenols when the waste materials were enriched with rock phosphate and ammonium sulphate.

In the second research, the OMW treatment with polyurethaneimmobilised *L. edodes* mycelium resulted in an overall abatement of phenolic and aromatic components as well as in an extensive decolourisation and in an apparent depolymerisation of the high molecular weight fraction. The best biodegradative results were obtained in the second batch but the dramatic decline in the decolourising ability of the immobilised biomass after the end of the third cycle indicated that the limit of the system's life-time should be 25 days. More recently, the same research group performed two interesting experiments on OMW using immobilised *L. edodes* (D'Annibale *et al.*, 1999; D'Annibale *et al.*, 2000; Hublik and Shinner, 2000). Previous studies on *Pleurotus ostreatus* (Palmieri *et al.*, 1994; Sannia *et al.*, 1994) showed that phenoloxidases immobilised in copper-alginate gel also resulted in an increase in the stability and activity of the entrapped enzyme in comparison with those of the free form. In fact, enzymes immobilised in alginate in the presence of Cu²⁺ exhibited a greater stability than that obtained with other divalent cations and the apparent half-time at 4°C was 30 days, while for the soluble form it was only 3 days.

Bioremediation in Fruit and vegetable processing industry

Industries that process fruits and vegetables are a very important part of the food industry especially in the Mediterranean countries where agriculture still remains one

of the main sources of income. The fruit and vegetable canning industry, the frozen vegetable industry, the vegetable dehydration industry, the fruit and vegetable drying industry, fruit pulping, tomato juice concentrate and fruit concentrate belong to this category.

These industries may operate seasonally since operation time depends on the production of the fruits and vegetable that they process. That means that the environmental pollution from those industries' waste will also be seasonal. According to the processing stage, different types of waste may be produced thus contributing with different percentages to the formation of the final process waste. The wastes from fruit and vegetable processing industries generally contain large amounts of solid suspensions and a high biochemical oxygen demand

(BOD). Some other parameters usually of interest to the waste treatment are pH, chemical oxygen demand (COD), dissolved oxygen and total solids. Indicative values for BOD, COD, suspended solids (SS) and pH for the processing of some fruit and vegetables are summarized (Table 4) (S.E. Tsiouris, personal communication). As has already been described, fruit and vegetable industry wastes consist of various by-products with an acidic pH (Riggle, 1989), and a moisture content of 80–90% (Grobe, 1994). The chemical composition of the wastes varies and depends on the processed fruit or vegetable. In general, the wastes consist of hydrocarbons and relatively small amounts of proteins and fat. The hydrocarbons are mainly sugars and nitrogen and cellulose fibers. The water wastes contain dissolved compounds, pesticides, herbicides and cleaning chemicals. These differences in the nature of the wastes require their separate treatment. Although the solid waste is mainly treated with composting, because of superior results slurry bioreactors and landfarming may also constitute two further options. A pretreatment is necessary to remove the water and neutralize the pH to ensure the best conditions for microbial growth and development. Bulking agents are also added to improve the porosity of the sludge and decrease the bulk density (Schaub & Leonard, 1996). The increased porosity may help in the drainage of water, which can be carried out either by gravity or by exerting pressure on the sludge. In some investigations the waste was left in open air so that the excess water evaporated (Grobe, 1994). The bulking agents employed include sawdust, paper, mature compost, straw, and coffee residuals. Of course, every industry prefers to employ easily available and, in particular, by-products of its own production. The bulking agents may appear more useful than just increasing the porosity because they can also increase the C: N ratios due to their high carbon content. Furthermore, the addition of bulking agents can affect the pH. It has been reported for example that the addition of pine sawdust and coffee grounds may increase the pH of fruit sludge. Additives that are used to raise the pH include wood ash and lime (Verville & Seekins, 1993). Aerated piles are more frequently used for the treatment of solid waste from fruit and vegetable industries (Nakata, 1994) because they allow the best mixing of the sludge while it is easy to add moisture, nutrients or more waste for processing if necessary.

However, if static piles are initially used, then later the compost has to be moved to an aerated pile for further cure.

Bioremediation in Dairy industry

Dairy industries contribute substantially to the pollution of

surface water and soil. The main wastes from these industries are chemically modified liquid wastes. The main characteristics of dairy waste can be summarized as follows:

- High organic load (fatty substances, etc.)
- Large variations in waste supply
- Considerable variations in pH (4.2–9.4)
- Relatively large load of suspended solids (SS) (400–2000 mg/l)

The dairy wastewater may contain proteins, salts, fatty substances, lactose and various kinds of cleaning chemicals. Detergents represent the biggest portion of chemicals used in dairies. The detergents may be alkaline or acid and are used for different purposes. Hydroxides or alkaline salts are responsible for the alkalinity of the detergent. They are mainly added to dissolve and remove proteins, but they also help to eliminate fats through saponification. Sodium hydroxide is the most widely applied alkaline detergent but for special applications it may be replaced or mixed with other strong bases. Acids are used to remove the inorganic deposits or so-called milkstone. For that purpose, nitric acid or phosphoric acid are used both alone and in combination. Both alkaline and acid detergents often contain additives to improve their cleaning capability. These are phosphates, sequestering agents, surfactants and some minor components like dispersing agents, anti-foaming agents and inhibitors (Romney, 1990).

However, detergents also present difficulties in their treatment. Wildbrecht (1990) reported that sodium carbonate passed a two-stage effluent treatment almost unchanged and was discharged into the river. Odzuk (1982) estimated that one-third of the sodium orthophosphate produced was utilized in biological wastewater treatment. On the other hand, EDTA used as a substitute for polyphosphates, has a low biodegradability and remains in the wastewater after treatment. Although fish are not poisoned, EDTA at 11 mg/l can inhibit algal growth (Schoberl & Huber, 1988). Apart from their undesirable foam production, leading to insufficient oxygen supply in activated sludge systems, surfactants were shown to affect strongly the ecosystems of rivers (International Dairy Federation, 1993). Some of them transform chlorophyll of the higher plants, whereas others are toxic to aquatic animals. Even the 'soft' surfactants often used today can disturb fish life when applied in high concentrations (Odzuk, 1982). What is noteworthy is that one of the surfactants' most important properties is their biodegradability. Still not all of them require the same treatment since some are degradable under aerobic and some under anaerobic conditions. On the other hand, not only biodegradability, but also toxicity has to be considered when the polluting effects of surfactants are investigated. Generally speaking, surfactants with greater biodegradability have higher toxicity (Maltz, 1988). An assessment of the various types of aerobic and anaerobic treatment systems employed in dairy waste processing was conducted (Bell, 1992). A partial denitrification and some uptake of phosphorus (from 40 to 70%) can be achieved in the activated sludge process. The application of chemical phosphate precipitation also increases because it allows an elimination of 80–90% of phosphorus (Schoberl & Huber, 1988).

Danforth (1992) [9] described in detail the use of an automatic computer control for a sequencing batch reactor (SBR) at another facility where the monitoring of pH and

dissolved oxygen (DO) allowed control of the system despite wide variations in flows and loading. A bench scale study of a fluidized-bed aerobic system yielded COD removals of 85 and 60% at loading rates of 500–900 g COD/ m³ h (Rusten *et al.*, 1992). These authors found that milk fat appeared to inhibit methanogenic activity and suggested that milk fat concentrations should be reduced to below 100 mg/l before anaerobic treatment. A laboratory-scale anaerobic sequencing batch reactor investigated by Sung and Dague (1995) attained a 90% reduction in soluble COD with a synthetic milk substrate.

Bioremediation in Meat and poultry industry

Meat, poultry and fish industries produce the highest loads of waste within the food industry. The meat industry contains slaughterhouses and processing units where meat is prepared, cut in pieces and is either frozen, cooked, cured, smoked or made into sausages. Slaughterhouses are more important than the other units in terms of environmental pollution. The wastes coming from these units contain various quantities of Food, fats, residues from the intestine, paunch grass and manure (Cournoyer, 1996).

The wastes are best separated into wastewater and solid waste. Solid waste, like intestines, pieces of meat or bones have been used as animal feed after further processing. Slaughterhouse wastewater is typically high in both moisture (90–95%) and nitrogen, has a high BOD and is odorous. Cooper and Russell (1992) published a summary of the treatment technologies and performance data of 44 meat processing plants in New Zealand, most of which were located in rural areas. Bulking agents are employed to make the waste sufficiently porous for aeration and to lower the moisture content down to 60–75% as a function of carbon source. In some cases where high-carbon bulking agents were needed, the compost required the addition of inorganic nitrogen to optimize the C: N ratio (Ross & Valentine, 1992). Pre-treatment is also necessary because the sludge derived from processing of wastewater contains pathogens. Therefore proper management is a prerequisite to ensure that potentially high levels of pathogens are eliminated (Cournoyer, 1996). Poultry wastes are equally problematic to meat wastes because the main source of wastewaters is the slaughtering process Starkey (1992).

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