Bioautomation, 2004, vol. 1, pp. 83 - 98

Utilisation of Food and Woodworking Production By-products by Composting

Uldis Viesturs[1,2], Dzidra Zarina[2], Silvija Strikauska[3], Andrejs Berzins[2]

[1]*Latvian State Institute of Wood Chemistry, 27 Dzerbenes Str., LV-1006 Riga, Latvia*
E-mail: koks@edi.lv

[2]*Institute of Microbiology and Biotechnology, University of Latvia, 4 Kronvalda Boul., LV-1586 Riga, Latvia*
E-mail: dz@lanet.lv

[3]*Latvian University of Agriculture, 2 Liela Str., LV-3001 Jelgava, Latvia*
E-mail: aazl@cs.llu.lv

Abstract: *The purpose of the study was to develop laboratory-scale technologies for composting milk/cheese whey, spent liquor, brewery yeast, fish processing by-products, etc., adding these by-products and special microorganism associations to the basic material - sawdust, bark, etc., also arranging different experimental composting sites.*

Two Trichoderma strains (Tr. lignorum, Tr. viride) and a nitrification association for regulating the circulation of nitrogen-ammonification and nitrification processes were applied. Monitoring of the composting quality was realised by microbiological and chemical analyses, and biotests for compost quality (toxicity) assessment. For purifying the polluted air from the composting facilities, the biofiltration technique was realised in a modified SSF system. Biodegradation of ammonia was investigated in a two-stage system with the inert packing material - dolomite broken bricks, and hemoautotrophic microorganisms: DN-1 (Pseudomonas sp.), DN-2 (Nitrosomonas sp.), DN-3 (Nitrobacter sp.) and DN-13 (Sarcina sp.). For hydrogen sulphide biodegradation, Thiobacillus thioparus-3 was immobilised on glass bricks as the carrier material.

Biodegradation efficiency of hydrogen sulphide was 87%. Biodegradation of ammonia in the first step in the two-stage system reached 77%, degradation of the gas remaining in the second step was 75%. Compost's quality was similar to black soil – brown-coloured, with good soil odour and without toxic compounds.

Keywords: *composting, food/woodworking by-products, biofiltration*

Introduction

From year to year, the world's population is increasingly ascertaining that our major wealth is renewable biomass, i.e. renewable resources of organic substances, which bind every year the atmospheric carbon in photosynthesis processes.

A considerable amount of photosynthesis systems is compensated by huge hydrolytic systems, which are formed mainly as a result of the activity of microbial enzymes worldwide (Fig. 1).

In nature, plant waste degradation proceeds slowly. Thus, for example, the degradation of plant waste in soil can proceed several weeks and months.

A different matter is bioconversion in reactors, where processes are controlled.

The vegetable kingdom's raw materials are divided into soluble and insoluble compounds. Therefore, liquid and solid state bioconversion (SSB) of waste, respectively, is developed.

In SSB, from plant waste such as straw, etc., fermenting it with definite microorganisms, e.g. *C. versicolar*, it is possible, for example, to obtain protein, necessary in cattle-breeding [15].

The analysis of the literature and our own experiments [15, 16, 11] demonstrates a technical possibility to realise the SSF or mixed SSF-SF process. However, the aerobic controlled SSF and SF processes of lignocellulose raw materials, particularly prevailing for delignifying cultures, are energetically unfavourable for microorganisms. Therefore, to ensure an intensive growth, the addition of energy rich substrates (ethanol, glucose, acetate, etc.) is necessary. This is evidently one of the reasons why a considerable delignification degree in SSF may be achieved only in long prosesses. Besides, the application of cellulose – or/and hemicellulose, i.e. assimilation cultures requires the complex treatment of raw materials (biomass delignification). Hence, the direct bioconversion process (direct cultivation) appears, in general, to be rather complicated, and the end products rather expensive.

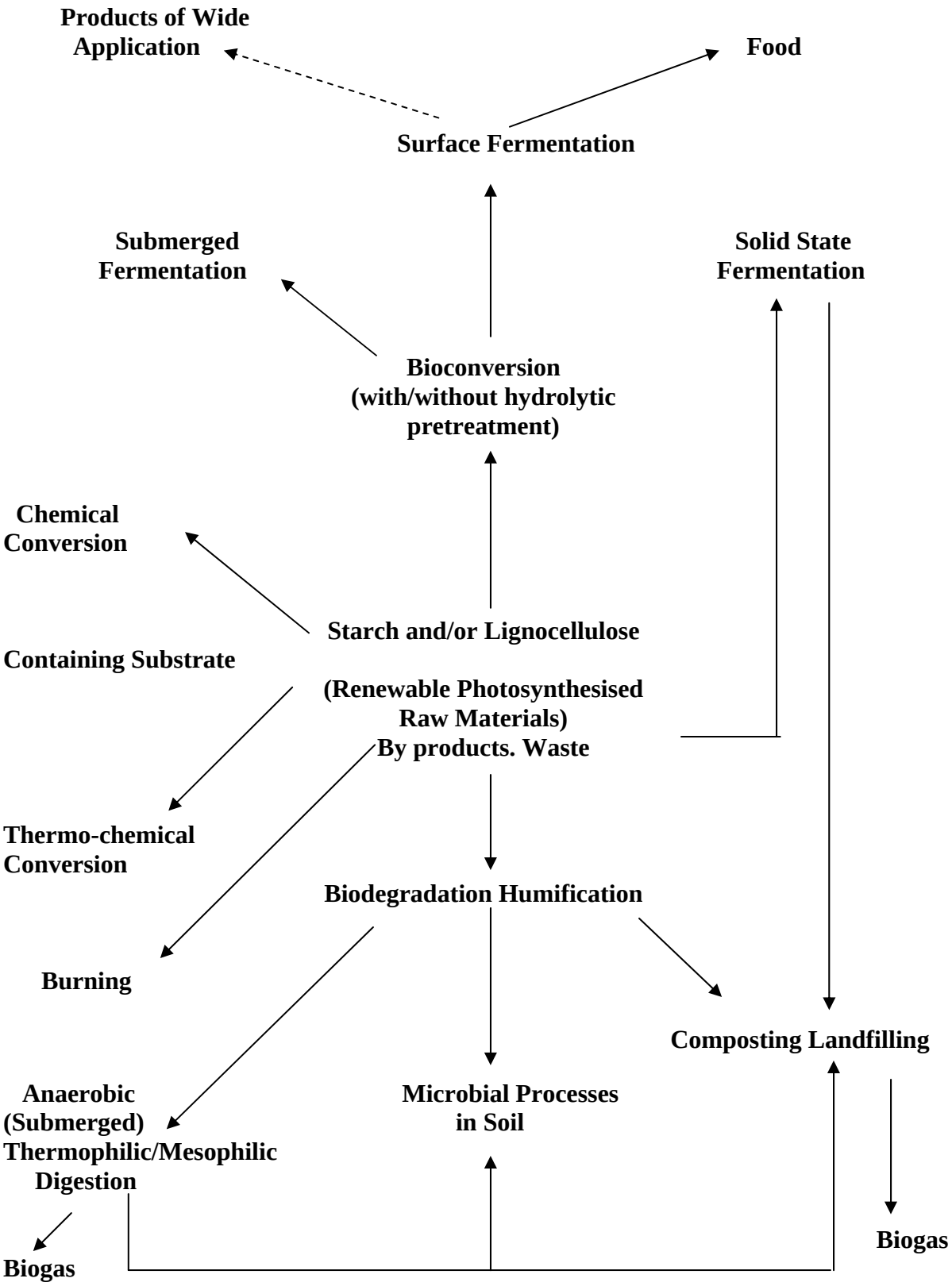

Fig. 1 Flow diagram of the conversion of photosynthesised biomass, by-products, waste, including their transformation under thermal conditions.

However, unfortunately, the acid hydrolysis of wood, agricultural waste, etc. is also currently rather expensive. The enzymatic hydrolysis is both expensive and hard-to-realise on an industrial scale.

Several chemical processes are used such as separation, gasification, liquefaction, etc. However, taking into account the fact that natural resources (oil, gas, etc.) are constantly decreasing, we should consider the photosynthesis, including in artificial plantations, and large-scale conversion of natural polymers (starch, hemicelluloses, cellulose) into monomers as the major way to obtain raw materials for the chemical, microbiological and other industries. In the future, the industrial-scale energy ethanol production under the enzymatic hydrolysis of special cultures grown in plantations, including fast-growing trees, is planned.

Our modest experience and the analysis of the world's literature show that, for such large capacity photosynthesised polymer conversion, SSF (as well as SF) is doubtful to prove itself economical [2, 3, 6]. The major application of SSF techniques may be in the output of comparatively expensive small amounts of microbial synthesis products for medicine, the food industry, agriculture, etc. For such purposes, the majority of SSF-SF variants may be used for practical applications.

To summarise the aforementioned, we consider mainly the following versions of the industrial aerobic SSF process:

1. Immobile or mixed (like in the Koji process) thin layers for comparatively expensive products.
2. Mixed specially tailored bacterial cultures with a low shear sensitivity.
3. Bioscrubbers for gas decontamination.
4. Composting of solid waste and other organic materials.

In the SSF fermentation process, the waste of the vegetable kingdom and organic origin can be also successfully converted to a compost rich in humic substances.

Composting, a very old biologically based technology, could be applied to wastewater sludge treatment. However, over the last several decades, it has not gained significant attention, because of the lack of a scientifically based knowledge for its application. At the same time, SSB, which is regarded as a promising novel low-cost bioremediation [13], allows a more

rational use of the selected microbial strains together with appropriate parameter settings to promote the growth and bioconversion [4].

The basic principles in composting and SSB are similar. However, in recent years, researchers have focused their attention on applying the SSB approach to optimise the composting process for the treatment of wastewater sludge. For an efficient degradation of wastewater sludge and wood waste, fungi and microorganisms' associations should be selected and then, for adjusted metabolic activities and microbial growth, the C:N ratio should be found.

Microorganisms can degrade the organic carbon present in the composting substrate only when they have enough nitrogen sources for growth.

In the case of nitrogen deficiency (the C:N ratio is too high), the composting process is inhibited. The availability of organic carbon is also crucial. Thus, taking into account the fact that the organic carbon obtained in fine disperse wood waste will be more rapidly utilised by microorganisms than that of coarsely shredded wood, specially adapted microbial associations can be applied in order to optimise the C:N ratio upon composting the waste of different dispersity.

The goal of this work was to select splitters and nitrification associations as well as to develop composting regimes and technologies. An attempt was made to develop an effective, rapid and controllable process that converts organic waste into high-quality soil improvers or bio-fertilisers and a complex biofiltration system for removal of H_2S and NH_3 from the composting facility waste gas in a solid state reactor.

Materials and methods

Realising of composting processes

Composting experiments were realised in the modified SSF system: both fermentation stages - anaerobic and aerobic were carried out in the same equipment [17, 19]. The ***specific objectives*** of this work were to use cheese whey from a dairy factory (1st variant), brewer's yeast biomass from a brewery factory (2nd variant), sewage sludge (3rd variant) from the wastewater treatment plant "Daugavgriva" in Riga, sewage sludge + brewer's yeast, sewage

sludge + cheese whey, sewage sludge + new batch of brewer's yeast as well coniferous and/or deciduous solid waste – sawdust (separately or in mixture).

Bioreactors and packing material

The biofiltration system for hydrogen sulphide removal from the composting facility waste gas was realised in a 3 l reactor (Fig. 2). The sulphide oxidation bacteria *Thiobacillus thioparus* sp.–5 was isolated from the biological activated sludge and immobilised on glass bricks as the carrier material. Also dolomite broken bricks, 20-30 mm in size, were used as the packing material. The two-stage system is shown in Fig. 3 and described in detail [10, 18].

Biodegradation conditions for waste gas

The pH range for isolated microorganisms was 6.0-8.5. These microorganisms allowed the operation of the bioreactor under conditions where moisture and pH could be less stringently controlled. During the microorganims' immobilisation and the adaptation period, mineral nutrients were continuously pumped through the filter bed.

The ammonia removal efficiency (RE) and biological elimination capacity (EC) were used for evaluation of the biodegradation process.

Cultures used

Microorganisms: 5 bacterial strains, designated DN-1 (*Pseudomonas* sp.), DN-2 (*Nitrosomonas* sp.), DN-3 (*Nitrobacter* sp.), N-13 *(Sarcina* sp.) and *Thiobacillus thioparus*-5 were isolated from the biological active sludge and used in the ammonia and hydrogen sulphide biodegradation.

The aqueous medium used for cultivation of the association for ammonia degradation was: $(NH_4)_2SO_4$ - 1.0 g, K_2HPO_4 - 2.0 g, $MgSO_4 \cdot 7H_2O$ - 0.5 g, $FeSO_4 \cdot 7H_2O$ - 0.001 g, $CaCO_3$ - 10 g, H_2O - 1000 ml, (pH 7.0-7.8). The same medium without ammonium sulphate was used for filter bed humidification in the one stage system, while 1% glucose was used for the two-stage biofiltration system.

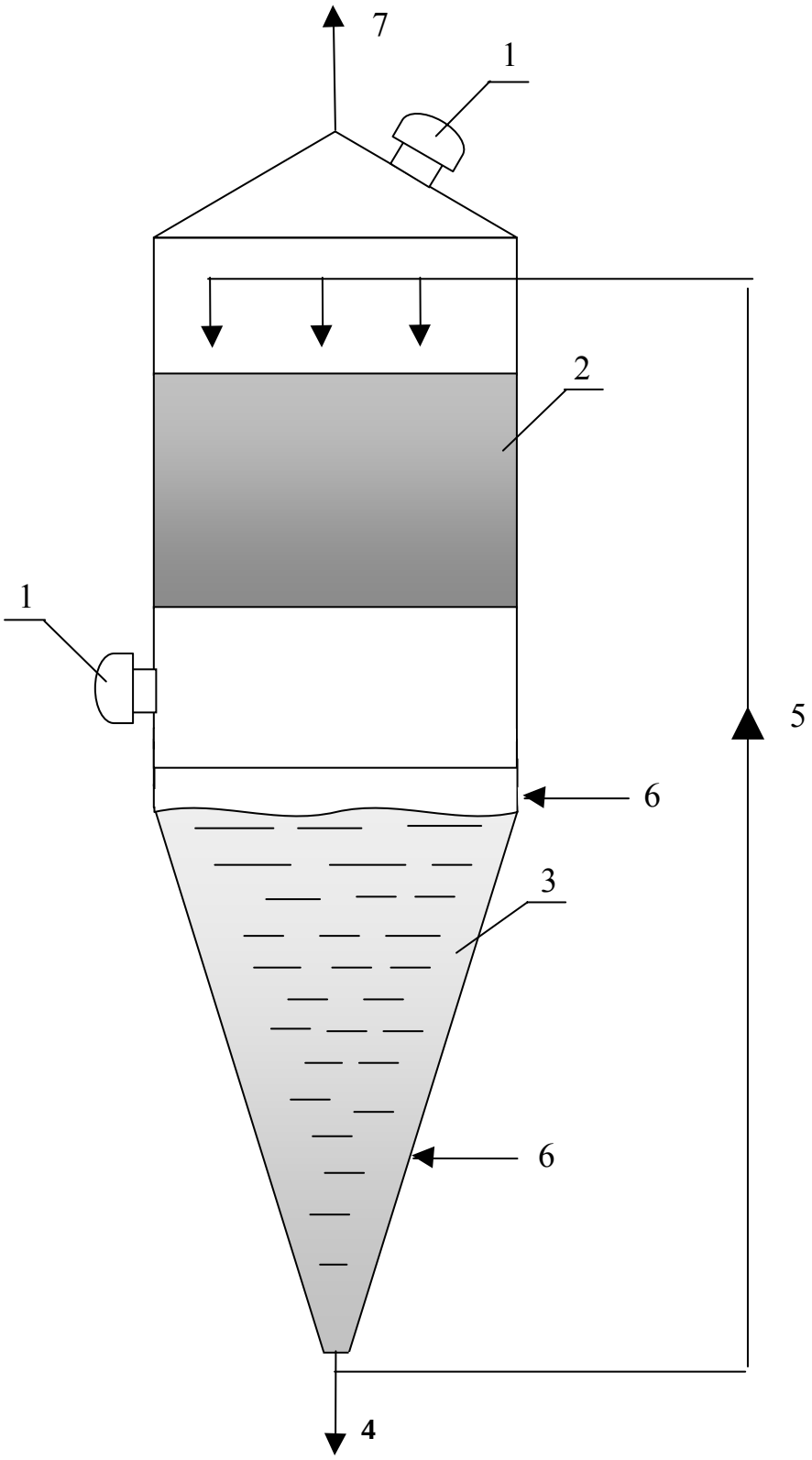

Fig. 2 Combined solid state/submerged bioreactor:

1 - hatch; 2 - packed bed; 3 - liquid/submerged bioreactor; 4 - outlet of the culture liquid;

5 - culture liquid for circulation; 6 - inlet gas; 7 - outlet gas.

The aqueous medium used for cultivation of *Thiobacillus thioparus*-5 and for humidification of the filter and glass bricks, was: $Na_2S_2O_3 \cdot 5H_2O$ - 5.0 g, NH_4Cl - 0.1 g, $NaHCO_3$ - 1.0 g, Na_2HPO_4 - 0.2 g, $MgCl_2 \cdot 6H_2$ - 0.1 g, H_2O - 1000 ml, (pH 8.0-8.5).

To promote the composting process, microorganisms' associations: *Tr. viride* and *Tr. lignorum,* and a nitrificator that regulates the circulation of nitrogen – the ammonification and nitrification processes, were applied as inoculum.

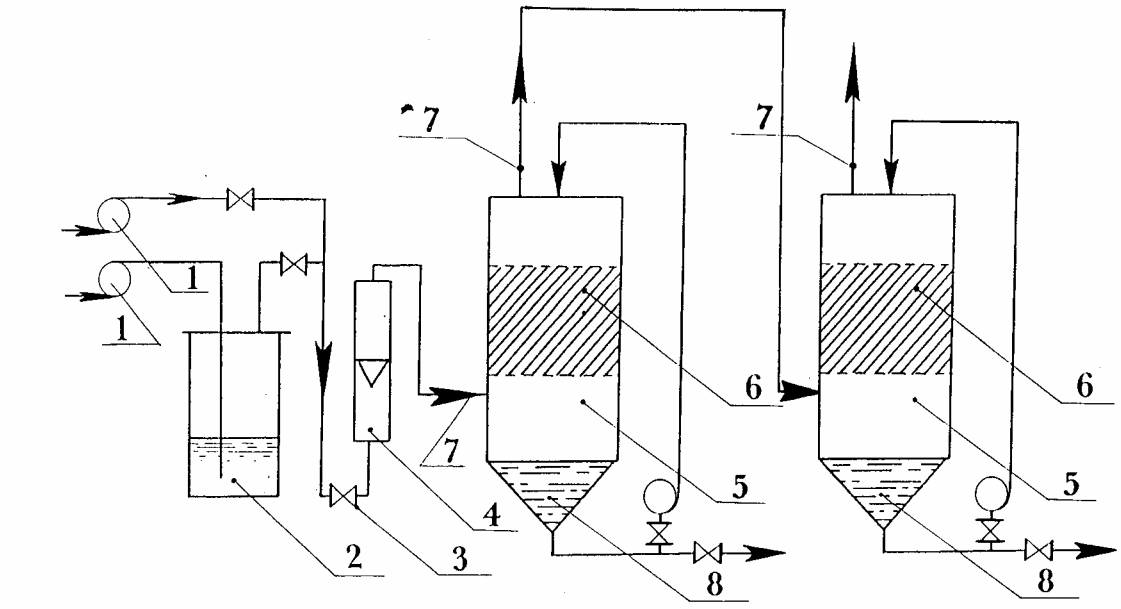

Fig. 3 Two-stage biofiltration system: 1 - compressor, 2 - vessel for contaminant under degradation; 3 - valve; 4 - measurement of volumetric flow rate; 5 - gas part of the bioreactor; 6 - biofilter; 7 - sampling points; 8 - submerged part.

Assessment of the compost quality

1) chemical analyses:

The concentrations of ammonia, nitrite, nitrate and hydrogen sulphide were determined by a FIAstar 5020 Analyzer. Total nitrogen was measured by the "Buchi" Kjeldahl Line. NO_3-N/NH_4-N. Total carbon was determined by the modified Tjurin's method [7] and C:N ratios were calculated.

2) microbiological analyses:

The plate count method was used for estimating the total number of microorganisms and fungi. The medium for quantification of the total number of bacteria was: Bacto nutrient agar

(Difco, USA), for fungi - Chapec medium, for *E. coli* - Endo agar, for *Salmonella* – agar Mak-Konky.

3) seeds germination tests with cress salad, cucumber and tomato.

4) microbiotests

The Rotoxkit F^{TM}, which measures the lethal effect of toxicants of rotifers freshly, hatched from cysts (with rotifers *Brachionus calyciflorus*), after 24-h exposure [8].

- Thamnotoxkit F^{TM} with *Thamnocephalus platyurus*, which measures the lethal effect of toxicants of crustacean freshly, hatched from cysts, after 24-h exposure [14].
- Ostracodtoxkit F with the benthos organism *Heterocypris incongruens* is a "direct contact" chronically toxicity microbiotest. After 6 days, the crustacean morbidity and growth intensity are compared with the control [5].

Results

Trichoderma lignorum was a more active splitter of cellulose and lignin substrates, while *Trichoderma viride* was mainly the producer of Trichodermin, and also the splitter of lignocellulose [1, 12, 11].

After a 1.2-2 month composting of sewage sludge under anaerobic conditions at the thermophilic regime, the amount of *Escherichia coli* and *Salmonella typhimurum* was 0. After 1.5 months at the aerobic regime, the C:N ratio was 20–30. The seed germination tests with all biotests (cress salad, cucumber and tomato) were weak at the beginning of the composting processes. At the same time, in the Toxkit microbiotests, a high toxicity level was established. The compost toxicity during the composting process decreased; at the end of the process, the effect of toxicity in percentage (EP) obtained with each of the applied microbiotests showed that the quality of the compost from the bioreactor was slight acute hazard. Conventional biotests like seed germination were less sensitive than alternative Toxkit microbiotests (Tables 1 and 2).

Table 1 Compost toxicity determined in Toxkit Microbiotests

Variants	Microbiotests (EP %)			
	Thamnotoxkit	Rotoxkit	Protoxkit	Ostracodtoxkit
Sawdust	100.0	100.0	68.3	86.6
Variant 1	10.0	21.3	-	16.6
Variant 3	13.3	80.0	0	96.6
At the beginning of the composting process in windrow	100.0	73.3	-	43.3
Qualitative compost (variant 1)	12.1	3.3	-	13.3

Table 2 Compost toxicity determined by seeds germination tests

Variants	*Seeds germination*		
	Cress salad	*Tomato*	*Cucumber*
Control	100	90	100
Variant 1	100	90	100
Variant 2	100	90	100
Variant 3	95	95	90

The total amount of bacteria during the composting process increased, and their amount at the end of the process was $6x10^7$ cells per g of dry compost. At the same time, the amount of fungi decreased from $3x10^3$ to $3x10^2$ cells per g of dry compost (Figs. 4 and 5).

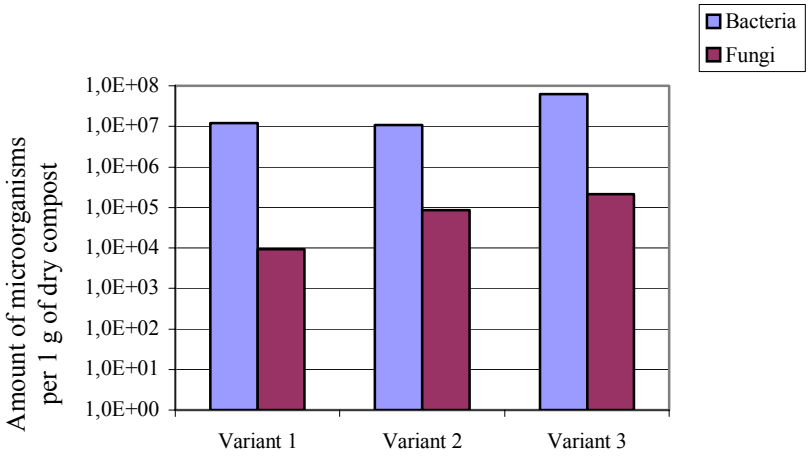

Fig. 4 Number of microorganisms in different composts

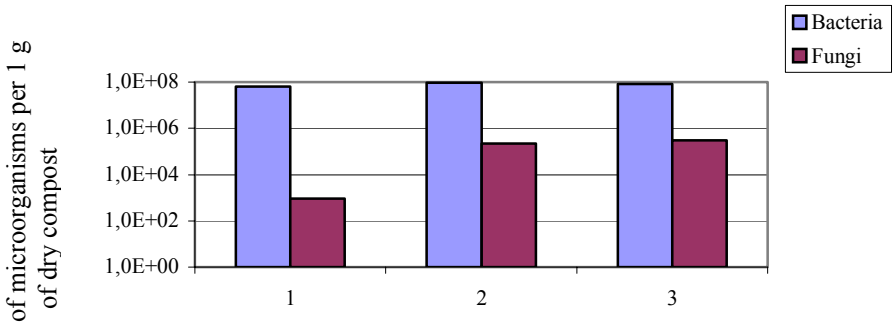

Fig. 5 Number of microorganisms in different composts

1 – at the end of composting process,
2 – in the middle of composting process,
3 – in the beginning of composting process

The biodegradation efficiency of hydrogen sulphide could be 97.1% at the gas flow rate 11.2 l/h (Table 3).

Table 3 Concentration of hydrogen sulphide before and after biofiltration

No	Gas flow rate (l·h)	Concentration of H_2S (%) (before biofiltration)	Concentration of H_2S (%) (after biofiltration)	Efficiency of bio-degradation (%)
1	20.0	0.023	0.019	17.4
2	14.6	0.033	0.021	36.4
3	11.2	0.016	0.006	62.5

The oxidation of ammonia was realised in a solid state reactor with the association of microorganisms that were isolated from biologically activated sludge. The ammonia concentration in the inlet gas was maintained at 0.2-0.5 g/m^3 (Table 4).

Owing to the high ammonia solubility, the pH was adjusted in the liquefied phase to 7.0. As a result of the biomass growing in the liquid phase of the reactor, NH_3 was metabolised in nitrites and nitrates, and the pH control was not necessary any more. The two-stage configuration was more effective for treatment of the waste gas containing a high amount of ammonia (Table 5).

Table 4 Formation of nitrification products and N-NH_4 during the ammonia biodegradation process

Inlet ammonia concentration ($g \cdot m^3$)	*Time (days)*	N-NO_2 ($g \cdot m^3$)	N-NO_3 ($g \cdot m^3$)	N-NH_4 ($g \cdot m^3$)
0.2	10	0.4	0	1.2
0.2	15	0.3	1.0	1.2
0.2	20	0.2	1.3	1.3
0.2	25	0.2	3.5	4.2
0.5	30	0.2	4.7	4.2
0.5	35	0.1	5.1	4.4
0.5	40	0.1	5.3	4.5

Table 5 Ammonia removal efficiency in one-stage biofiltration system

Experiment	Ammonia concentration in inlet gas ($g \cdot m^3$)	Ammonia concentration in outlet gas ($g \cdot m^3$)	Ammonia load ($g \cdot m^3$ h)	Biological elimination capacity ($g \cdot m^3$ h)	Removal efficiency (%)
1	0.5	0.01	0.41	0.33	98
2	1.2	0.10	0.97	0.87	92
3	2.0	0.30	1.62	1.13	85
4	2.5	0.50	2.03	1.20	80
5	3.0	0.71	2.43	1.20	74
6	4.1	2.20	3.30	0.75	46
7	5.0	3.12	4.05	0.50	37
8	7.0	5.63	5.67	0.34	20
9	5.0	3.2	4.05	0.01	35

The two-stage biodegradation system was more efficient not only for the total DE, but also increased the biological degradation level [9]. The considerable loss of nitrogen in this system was assumed to result from the denitrification process that occurred in the second biofilter.

Ammonia removal efficiency in two-stage biofiltration system

Biofilter 1 ammonia concentration ($g \cdot m^3$)		Biofilter 2 ammonia concentration ($g \cdot m^3$)		Removal efficiency (%)
Inlet gas	Outlet gas	Inlet gas	Outlet gas	
2.0	0.1	0.1	0	100
2.5	0.3	0.3	0	100
3.0	0.7	0.7	0	100
4.1	1.1	1.1	0.3	92.3
5.2	3.2	3.2	1.4	73.1
7.0	5.6	5.6	2.5	64.3

Discussion

As shown above, we have gone through and optimised the following stages of utilising by-products. Organic waste (sewage sludge, food waste, brewery and ethanol factory waste and sawdust) was processed using a horizontal 3 l laboratory reactor. The reactor was specially designed, since both fermentation stages, i.e. anaerobic and aerobic, were carried out in the same equipment.

The first step of the biodegradation process can be realised at the anaerobic thermophilic regime, providing the necessary mixing of the compost.

The second step of the biodegradation process should proceed at the mesophilic aerobic regime, providing an intensive aeration of the compost. The application of specific adapted microorganisms' associations in aerobic composting processes provides effective and rapid composting of waste [19]. However, for large-scale processing, windrows seem to be most appropriate.

The waste gas from the composting facilities contains different components, which create odour nuisance and acid rain.

The biofiltration system for removal of hydrogen sulphide from the composting facility waste gas was realised in a 3 l solid state reactor. The sulphide oxidation bacteria *Thiobacillus thioparus* sp.–5 were isolated from the biological activated sludge and immobilised on glass bricks as the carrier. The biodegradation efficiency of hydrogen sulphide amounted to 97.1% at the gas flow rate 11.2 l/h.

The oxidation of ammonia was realised in a solid state reactor with 4 bacterial strains: *Pseudomonas* sp., *Nitrosomonas* sp., *Nitrobacter* sp., *Sarcina* sp. that were isolated from the activated sludge. The biofiltration system makes it possible to clean continuously the waste gas from the composting facility from ammonia with the degradation efficiency 97%.

However, after the consideration of the main feasibility aspects, first of all, the gathering of waste gases, the practical realisation of the system: windrows - biofiltration seems rather questionable.

Biofiltration could be recommended for treatment of more concentrated gases, like we had in the laboratory-scale system.

Conclusions

The targeted (to the type and composition of waste) formation of the culture's composition, performed under controlled regimes in SSF-SF bioreactors, followed by windrows, is recommended for utilising food and woodworking by-products.

The composting quality was controlled by chemical analyses, biotests – seed germination, microbiotests - for compost quality assessment (toxicity), and microbiological methods for detection of human and animal pathogenic agents (*Escherichia coli*).

The compost was with a good soil odour and without toxic compounds.

Abbreviations used

SSF – solid state fermentation

SF – submerged fermentation

SSB – solid state bioconversion

RE – ammonia removal efficiency

EC – biological elimination capacity
EP – effect of toxicity in percentage
DE – degradation efficiency, %.

Acknowledgements

This work, as the projects 01.389, 01.0370 and 01.369, was financed by the Latvian Council of Sciences.

References

1. Apsite A., U.Viesturs, V.Šteinberga, M.Toma (1998).Morphology and Antifungal Action of the Genus *Trichoderma* Cultivated in Geometrically Dissimilar Bioreactors, World J. Microbiol. Biotechnol., v. 14, pp. 23-29.
2. Berzins A., M.Rikmanis, M.Toma, U.Viesturs, S.Gonta (2001a).Cultivation of *Zymomonas mobilis* 113S at Different Mixing Regimes and their Influence on the Levan Formation, Acta Biotechnol., v. 21 (1), pp. 19-26.
3. Berzins A., M.Toma, M.Rikmanis, U.Viesturs (2001b).Influence of Micromixing on Microorganisms and Products, Acta Biotechnol., v. 21 (2), pp. 155-170.
4. Molla A.H., A.Fakhru'l-Razi, M.M.Hanafi, M.Zahangir Alam (2004). Optimization of Process Factors for Solid-state Bioconversion of Domestic Wastewater Sludge, International Biodeterioration and Biodegradation, v. 53, pp. 49-55.
5. OSTRACODTOXKIT F.: Cronic "Direct Contact" Toxicity Test for Freshwater Sediments. Standard Operation Procedure, Creasel, Deinze, Belgium, 2000.
6. Priede M.A., J.J.Vanags, U.E.Viesturs (2001). Performance of *Aspergillus niger* Cultivation in Geometrically Dissimilar Bioreactors Evaluated on the Basis of Morphological Analyses, Food Technol. Biotechnol., v. 40 (1), pp. 57-66.
7. Rinkis G.J., H.K. Ramane, T.A.Kunickaja (1987). Methods for Analysis of Soils and Plants, Zinatne, Riga, 174 pp. (in Russian).
8. ROTOXKIT F^{TM}: Freshwater Toxicity Test with Rotifer. Standard Operation Procedure, Creasel, Deinze, Belgium, 1992.
9. Schmidt D. (2002). Odor, Hydrogen Sulphide, and Ammonia Emissions from the Composting of Caged Layer Manure - Conference of the American Society of Agricultural Engineering, USA, 10 October 2000, pp. 1-9.

10. Strikauska S., D.Zarina, A.Berzins, U.Viesturs (1999). Biodegradation of Ammonia by Two Stage Biofiltration System, Environmental Engineering and Policy, v. 1 (3), pp. 175-179.
11. Telysheva G., T.Dizhbite, G.Lebedeva, G. Rossinskaja, V.Jurkjane, O.Treikale, U.Viesturs, M.Daugavietis (2002). Lignin-based Products Stimulating Soil Phytoremediation, Acta Biotechnol., 22 (1-2), pp. 167-173.
12. Telysheva G., G.Lebedeva, T.Dizhbite, N.Zaimenko, J.Ammosova, U.Viesturs (2000). Use of Silicon-containing Lignin Products for *in situ* Soil Bioremediation, In: Wise D.L., D.J.Trantolo, E.J.Cichon, H.I.Inyang, U.Stottmeister (Eds.), Bioremediation of Contaminated Soils, Marcel Dekker, Inc., New York – Basel, pp. 699-727.
13. Tengerdy R.P. (1992) Solid State Ferementation of Lignocelluloses, In: Doclle, H.W., Mitchel, D.A., Rolz, C.E. (Eds.), Solid Substrate Cultivation, Elsevier, London, pp. 269-282.
14. THAMNOTOXKIT F^{TM}: Freshwater Toxicity Test with Crustaceans. Standard Operation Procedure, Creasel, Deinze, Belgium, 1995.
15. Viesturs U.E., M.P.Leite (1996). Physiological and Technico-engineering Aspects of Lignocellulose Solid-state Fermentation with Filamentous Fungi, In: Advances in Bioprocess Engineering, Kluwer Academic Publishers, Dordrecht – Boston – London, pp. 81-87.
16. Viesturs U., M.Leite (1997). Certain New Biotechnological Processes and the Equipment for their Implementation (Review), Appl. Biochem. Microbiol., v. 33 (3), pp. 213-235.
17. Viesturs U., Dz. Zarina, S.Strikauska, A.Berzins, A.Zilevica (2002). Solid State Systems for Bioremediation and Biodegradation - VI International Symposium on Environmental Biotechnology and IV International Symposium on Cleaner Bioprocesses and Sustainable Development, Mexico, Veracruz, 1.2 pdf (CD).
18. Viesturs U., Dz.Zariņa, S.Strikauska, L.Dubova, A.Bērziņš (2003). Emission of Odour Gases during the Composting Processes of Different Organic Wastes - European Congress on Biotechnology, Basel, Switzerland, 24-29 August, p. 129.
19. Zariņa Dz., F.Utināns, Method for Biocomposting of Organic Waste in Field Conditions (Paņēmiens Organisko Atkritumu Biokompostēšanai Atklātā Laukā), Latvian Patent No. 13022, 20.09.2003 (filed 19.12.2001) (in Latvian).