ARCHIVES OF ENVIRONMENTAL PROTECTION

vol. 37 no. 3 pp. 15 - 19 2011

PL ISSN 2083-4772

USABLE PRODUCTS FROM SEWAGE AND SOLID WASTE

MARIA ŁEBKOWSKA*, MONIKA ZAŁĘSKA-RADZIWIŁŁ

Warsaw University of Technology, Faculty of Environmental Engineering,
Department of Biology
Nowowiejska 20, 00-653 Warsaw, Poland
*Corresponding author's e-mail: maria.lebkowska@is.pw.edu.pl

Keywords: Microbial biomass protein, food from wastewater, lipases.

Abstract: Sewage and solid waste can be a valuable source of materials used directly or indirectly in manufacturing of usable products. These processes are associated with elimination of pollutants from liquid and solid wastes. The best-known methods of waste management are production of biogas and composting. This paper focuses on the possibility of obtaining biomass as a source of protein feed (whose value, in terms of the composition of aminoacids and microelements, is comparable with conventional feed, e.g. soymeal, bonemeal or fishmeal). Sewage components for bacterial, fungal, algal and vascular plants' culture are characterized as a source of protein feed. Methods of industrial scale production of enzymes, mainly proteases and lipases that have broad applications in various industries, are discussed. Development perspectives of inexpensive methods of usable products from waste production are showed. Interdisciplinary nature of presented issues, which requires cooperation of specialists in biology, chemistry and technology, is emphasized.

INTRODUCTION

Sewage and solid waste can serve as a source of usable products, among which the most renowned are biogas and compost, and more recently polihydroxyacids, biological surfactants and components of biofuels. Among other products the role of biomass as a protein supplement in feed and enzymes used as industrial additives in detergents in leather and textile industry should be emphasized.

Protein feed extracted from sludge are biomass of bacteria and yeast called Single Cell Protein (SCP) and biomass of fungi and algae. Biomass production is often associated with simultaneous sewage treatment. Several substrates can be used in biomass cultivating, e.g. molasses, whey, effluent from baker's yeast culture, sulfite waste liquors from cellulose production, stillage, wastes from soy protein production, starch hydrolysates from wheat, maize, potatoes and rice straw processing, brewing and sugar waste, wastes from the wool laundry, from chitin and chitosan production, from fish processing industry as well as wastes containing hydrocarbons. The main substrates used by microorganisms are sugars and other simple organic compounds.

MICROORGANISMS AS A SOURCE OF PROTEIN FEED

Cellulomonas and *Alcaligene*s are the most commonly used bacterial genera for SCP production. They are also methylothrophs growing on C_1–C_4 compounds [2]. Bacteria may be present in yeast cultures acting as mixed population of good nutritional values. *Candida*, *Hansenula*, *Torulopsis*, *Saccharomyces* and *Pichia* are among the most prevalent types of yeast for SCP production. As an example, *Candida arborea* AS1.257 biomass production on rice straw hydrolyzate was performed by Zheng *et al*. In their study 9l bioreactor with 1.1 l/min air flow at a temperature of 29^{o}C was used. After 48 hours the biomass of 19.8 mg/ml with protein content of 59.0% was obtained. Hydrolyzate sugar consumption was 76.1% [12].

Filamentous fungi such as *Aspergillus*, *Fusarium*, *Penicillium* can also serve as a source of protein feed. Jin *et al.* took sludge from starch production and obtained 7.5–9.2 g/l of biomass of *Aspegillus oryzae* and *Rhizopus oligosporus*. The product (obtained in non-sterile conditions) contained 45% protein. At the same time 95% decrease of BOD and COD and 75% reduction in nitrogen and phosphorus were observed [8].

Algal biomass is a source of not only protein but also of mineral salts and vitamins. Algae are cultivated in treated sewage exposed to light with the addition of CO_2. *Chlorella*, *Dunaliella*, *Spirulina* and diatoms should be mentioned among genera used in protein production. Recently researchers have been focusing on the usability of algal components as dietary supplements for humans. Guil-Guerrero *et al.* cultivated *Porphyridum cruentum*, *Nannochloropsis* sp. and *Phaeodactylum tricornutum* algae in sterile seawater aerated with CO_2 (0.0014 mol/s) under fluorescent lamp exposure. Analysis of nitrogen solubility, viscosity, absorption capacity in water and oil and emulsification properties revealed that the biomass has functional properties comparable to soymeal [6].

A review work of Anupama and Ravindra shows that microbial protein may constitute a feed supplement replacing costly conventional feeds, such as soymeal, fishmeal or bonemeal [2]. The composition of algal, fungal and bacterial biomass is presented in Table 1. Comparison of aminoacids content in *Aspergillus niger* protein with FAO standards (Food and Agriculture Organization of the United Nations) is showed in Table 2.

Table 1. Composition of microbial protein (selected data)

	Percentage composition of weight		
Component	algae	fungi	bacteria
Protein	40–60	30–70	50–83
Total N (protein + nucleic acids)	45–65	35–60	60–80
Lysine	4.6–7.0	6.5–7.8	4.3–5.8
Methionine	1.4–2.6	1.5–1.8	2.2–3.0
Fats/Lipids	5–10	5–13	8–10
Carbohydrates	9	ND*	ND
Nucleic acids	4–6	9.7	15–16
Mineral salts	7	6.6	8.6
Ash	3	ND	ND

*ND – no data

Table 2. Comparison of aminoacids content of *Aspergillus niger* protein with the FAO standards

	Aminoacids (%)							
	1	2	3	4	5	6	7	8
FAO	4.20	4.80	4.20	4.20	2.20	2.80	2.80	2.80
Aspergillus niger	4.36	6.80	3.75	4.50	0.35	5.70	traces	3.00

where:

1 - valine,	5 – methionine,
2 -leucine,	6 - phenylalanine,
3 - isoleucine,	7 – cystine,
4 - lysine,	8 – tyrosine.

The final protein product requires necessary research before marketing authorization. It refers mainly to the content of nucleic acids, toxins (mycotoxins) and chemical contaminants such as metals and PAHs, microbiology (pathogens) and parasitology (helminth eggs).

The content of nucleic acids should not exceed 3–6% (preferably 1.5%) due to their metabolism, leading to uric acid production. Their content may be reduced by reactions of enzymatic degradation or by use of a heat shock method. Bacterial exotoxins and endotoxins should be removed, e.g. thermally or chemically by alcohol, formaldehyde or dilute acids treatment. Mycotoxins production can be inhibited by elimination of genes responsible for their synthesis within fungal productive strains.

Recovery of proteins (such as blood or casein) directly from wastes should also be mentioned. Chen *et al.* determined the conditions for obtaining the protein from wastewater after chitin production. They added 2 ml of 1% chitosan solution in 1% acetic acid and 1% $FeCl_3$ to 50 ml wastewater and then carried out a four-hour flocculation. The protein precipitate was rich in exogenous aminoacids (isoleucine, methionine and lysine) [3]. Protein recovery from fishmeal wastewaters by ultrafiltration was evaluated for a production of 544 tons/y of fishmeal with 66% protein content [1].

ACQUISITION OF ENZYMES

Protease and lipase should be noticed among the enzymes which can be extracted from the microbial culture. There are approximately 200 enzymes in the commercial use. Their biosynthesis takes place in bioreactors and they are obtained by exoenzymes isolation from the environment (hydrolases) or endoenzymes isolation from cells (e.g. oxidoreductases). Wastewater from food industry, waste oils, molasses, beet pulp, starch, wheat bran, sewage sludge are used as media for the production of enzymes aimed for industrial purposes. Thermostable proteases used in the manufacture of detergents and in the tanning industry can be extracted from the thermophilic bacteria culture on sludge as a medium. Chenel *et al.* isolated *Bacillus licheniformis* which was cultured in a fermenter with activated sludge at a concentration of 25 g/l of suspended solids at a temperature of 55°C. Under semisynthetic medium conditions 10^9–10^{10} cfu/ml of bacteria was obtained and the activity of proteases against casein was 3.2–5.3 IU/ml. The authors calculated that in the annual production of proteases added to detergents (4.25 x 10^8 kg/year) 234.25 tons of dry sludge may be used [4].

Lipases as biocatalysts decomposing fats and oils (or engaged in organic synthesis) are widely used in industry including:

– transesterification processes in the production of cocoa butter, milk powder, unsaturated fatty acids and biodiesel from vegetable oils;
– production of biodegradable polymers (of polyesters character);
– the textile industry, among others for wetting the polyester fibers and obtaining antistatic properties;

– production of detergents for contaminants elimination from fabrics;
– leather industry for fat elimination from leather;
– paper industry for triglycerides and wax removal from paper pulp;
– treatment of pipes and sewers [11].

Among the microorganisms used for industrial lipases production the most important are: *Bacillus*, *Pseudomonas*, *Burkholderia*, *Streptomyces*, *Aspergillus, Rhizopus, Penicillium, Mucor, Geotrichum, Candida, Pichia, Saccharomyces*, and *Torulospora*.

After the culturing process enzymes are recovered from the supernatant after biomass centrifugation or by ultrafiltration. The lipases are then treated in a manner appropriate to their application. They can be immobilized on hydrophobic media.

Lipases as thermostable enzymes have many advantages:
– are characterized by a significant rate of diffusion;
– accelerate solubility of lipids and hydrophobic compounds in water;
– reduce viscosity;
– reduce the risk of microbial contamination [7].

Liu *et al.* conducted a study on the optimization of conditions for lipase production by *Burkholderia* sp.C20. The authors found that the choice of temperature, pH, concentration of olive oil (as a substrate) and the content of $CaSO_4$ and NaCl increased enzyme activity 5-fold (from 0.8 to 3.9 U/ml) [10]. Lara and Park demonstrated the usefulness of wastewater from oil refining as a substrate for the production of esters with the involvement of lipase form *Candida cylindracea*. At the same time they showed that the organic compounds adsorbed on the bleaching earth were completely decomposed in the process of biological treatment. The results showed the possibility of utilization of wastes from the production of vegetable oils in order to produce alkyl esters of fatty acids for biodiesel [9].

Yeast cells contain numerous enzymes, namely proteases and pectinases, among others. Thus the industrial production of these enzymes, especially from brewer's yeast is a field to explore. Yeast also may serve as suitable biosorbents to remove heavy metals and dyes from wastewater [5].

The perspective of receiving useful products from sewage and solid wastes was discussed in this paper. This strategy enables both wastewater treatment and production of additional products, leading to elimination of expensive, "pure" biotechnologies. The use of waste materials as substrates for microorganisms reduces the possibility of environment pollution and/or their concentrations.

REFERENCES

[1] Afonso M.D., J. Ferrer, R. Bórquez: *An economic assessment of protein recovery from fish meal wastewaters by ultrafiltration*, Desalination, **165**, 281 (2004).

[2] Anupama, P. Ravindra: *Value-added food: Single cell protein,* Biotechnology Advances, **18**, 459–479 (2000).

[3] Chen X., C. Li, X. Ji, Z. Zhong, P. Li: *Recovery of protein from discharged wastewater during the production of chitin*, Bioresource Technology, **99**, 570–574 (2008).

[4] Chenel J.P., R.D. Tyagi, R.Y. Surampalli: *Production of termostable protease enzyme in wastewater sludge using thermophillic bacterial strains isolated from sludge,* Water Science and Technology, **57**, 639–645 (2008).

[5] Ferreira J.M.P.L.V.O., O. Pinho, E. Vieira, J.G. Tavarela: *Brewer's Saccharomyces yeast biomass: characteristics and potential applications,* Trends in Food Science and Technology, **21**, 77–84 (2010).

[6] Guil-Guerrero J.L., R. Navarro-Juarez, J.C. Lopez–Martinez, P. Campra–Madrid, M.M. Rebolloso–Fuentes: *Functional properties of the biomass of three microalgal species*, Journal of Food Engineering, **65**, 511–517 (2004).

[7] Hasan F., A.A. Shah, A. Hameed: *Industrial applications of microbial lipases,* Enzyme and Microbial Technology, **39**, 235–251 (2006).

[8] Jin B., X.Q. Yan, Q. Yu, J.H. van Leeuwen: *A comprehensive pilot plant system for fungal biomass protein production and wastewater reclamation,* Advances in Environmental Research, **6**, 179–189 (2002).

[9] Lara P.V., E.Y. Park: *Potential application of waste activated bleaching earth on the production of fatty acid alkyl esters using Candida cylindracea lipase in organic solvent system,* Enzyme and Microbial Technology, **34**, 270–277 (2004).

[10] Liu C.H., W–B. Lu, J–S. Chang: *Optimizing lipase production of Burkholderia sp. by response surface methodology*, Process Biochemistry, **41**, 1940–1944 (2006).

[11] Sharma R., Y. Chisti, U.C. Banerjee: *Production, purification, characterization and applications of lipases*, Biotechnology Advances, **19**, 627–662 (2001).

[12] Zheng Y-G., X-L. Chen, Z. Wang: *Microbial biomass production from rice straw hydrolysate in airlift bioreactors*, Journal of Biotechnology, **118**, 413–420 (2005).

Received: April 11, 2011; accepted: June 20, 2011.

PRODUKTY UŻYTECZNE ZE ŚCIEKÓW I ODPADÓW STAŁYCH

Ścieki i odpady stałe mogą być cennym źródłem surowców wykorzystywanych bezpośrednio lub pośrednio do wytwarzania użytecznych produktów. Procesom tym towarzyszy eliminacja zanieczyszczeń z odpadów płynnych i stałych. Do najbardziej znanych sposobów zagospodarowania odpadów należy produkcja biogazu i kompostowanie. W niniejszej pracy zwrócono szczególną uwagę na możliwość pozyskiwania biomasy jako źródła białka paszowego, którego wartość pod względem składu aminokwasów i mikroelementów jest porównywalna z paszami konwencjonalnymi, między innymi z mączką sojową, kostną, rybną. Scharakteryzowano surowce ze ścieków do hodowli bakterii, grzybów, glonów i roślin naczyniowych jako źródeł białka paszowego. Omówiono sposoby produkcji na skalę przemysłową enzymów, głównie proteaz i lipaz mających szerokie zastosowanie w różnych gałęziach gospodarki. Wskazano perspektywy rozwoju metod wytwarzania użytecznych produktów z odpadów, eliminujących drogie technologie. Podkreślono interdyscyplinarny charakter przedstawionej problematyki, wymagającej współdziałania specjalistów z zakresu biologii, chemii i technologii.