Keywords Biosorption; Heavy metal; Side stream; Microbe; Yeast; Fungi; Bacteria; Saccharomyces; Aspergillus; Bacillus; Streptomyces; Trichoderma

Introduction

Heavy metals belong to the most problematic pollutants at various

Yeast fermentations

Ethanol fermentation process consists of several unit operations to convert carbohydrates into ethanol by *S. cerevisiae*. is technology is employed worldwide in production of alcoholic beverages and bioethanol. A er brewing the yeast cells are collected from the bottom of tank or separated by centrifugation to form slurry of 20-50% solids [6]. Loose slurries are further concentrated by ltration a er which they are thermally disrupted at around 80°C and optionally also with organic acids. e production capacity of major Finnish breweries is over 400 million l/a [7] and the respective quantity of formed yeast containing residues is approximately 2000 t/a.

Bioethanol is produced by St1 Biofuels Oy in quantity of 13,000 t/a in Lahti, Vantaa, Hamina, Jokioinen and Hämeenlinna. Ethanol is further concentrated at Hamina. ese production sites produce altogether over 75,000 t/a of residues that contain also yeast cells. Hämeenlinna plant directs all organic residues to biogas production [8] while the other plants produce also animal feed. e amount of produced feed is over 45,000 t/a [9-12]. e company has also been appointed an environmental permit in 2013 for production of lignocellulose ethanol in Kajaani [13]. Yeast residues of the process together with other residues would be directed to energy production.

Enzyme production

e main steps in enzyme production processes are medium preparation, inoculum preparation, inoculation and fermentation, cell removal, product puri cation and concentration, and formulation. e nature of produced enzyme a ects also properties of microbial residues. Extracellular enzymes are recovered from the fermentation broth a er removal of cells which remains the cells intact. Cell removal is conducted using lter press together with lter aids and occulants. On the contrary, intracellular products are recovered via disruption of cells by chemical or mechanical methods, a er which cell debris are included in the residues.

Currently industrial enzymes are manufactured in Finland by two companies, Genencor and Roal Oy. Both companies occupy lamentous fungi and Gram-positive bacteria in their processes [14]. Genencor, owned by DuPont, has production sites at Jämsänkoski and Hanko. According to operative environmental permission appointed by authorities, 90% of the residues from Hanko plant comprise of microbial cell side stream which accounted approximately 16,000 t/a in 2002 [15]. Jämsä plant residues account to approximately 8,000 t/a microbial cell mass. Residues are currently used for biogas production and also composting has been considered.

Roal Oy, owned by Associated British Foods, has a production site at Rajamäki. e production capacity of the plant is 10,000 t/a, from which approximately half is in use. Microbial cell residues, among some diatomite, account to approximately 2,100 t/a in which solid content is some 40%. From this amount 1,500 t/a are directed to combustion in order to secure trade secrets related to microbial strain development, and the rest 600 t/a to composting [16].

Other processes

Suomen Hiiva Oy, owned by Lallemand, produces approximately 8,000 t/a baker's yeast in Lahti, the total production capacity being 12,000 t/a. Yeast is produced in batch processes by sequential scalin from 200 g inoculum to 200 t of culture within one week [17]. According to the interview and environmental permit, the plant does not produce signi cant amounts of cell waste [17].

Finnish based company Neste Oil has been developing microbial oil production processes for the manufacturing of biodiesel. e company has operated a pilot plant for producing microbial oil from waste and residues at its site in Porvoo, Finland, since 2012 althoug the project is currently on hold. In principle, microbe oil is produced via accumulation of lipids to fungal and yeast cells utilizing plant biomasses as feedstocks. Also, genetically modi ed bacteria have bee developed for the purpose [18].

Cursor Oy, the Kotka-Hamina Regional Development Company, has developed a process that utilizes forest industry side streams as substrates for micro algae in order to produce a variety of products [19]. In this process the algal cells would be valorized comprehensively and thus their use for biosorption applications would not be possible.

Total biomass potential

e investigated companies produce altogether over 103,000 t/a of microbial side streams. is value represents all material in the streams and thus the actual quantity of cellular biomass is lower. Exact quantities of cell material cannot be determined without further characterization of each stream. However, some estimation can be made based on the environmental permit documentations. e majority of the side streams is formed in brewing and bioethanol production and accordingly, yeast *Saccharomyces cerevisiae* is the predominant organism in the stream e produced side streams are typically directed to biogas plants and to feed production. Minor portion is combusted due to trade secrets.

Cell wall characteristics of microbes in side streams

e structure of cell walls is a major factor regarding the biosorption capacity of microbial biomass. e cellular composition tends to be rather similar between organisms within the same genus or order [20] while di erences in structure can be found between eukaryotic and prokaryotic organisms, and between Gram-positive and Gramdiameters above 50 µm have been observed. Regarding the cell wall structure most bacteria can be divided into Gram-positive or Gram-negative cells based on their response to Gram-staining. Cell surface, structurally and chemically, is more complex in Gram-negative bacteria while the surface in Gram-positive bacteria is composed mostly from peptidoglycan (Figure 2) [21]. The most common representatives of Gram-positive bacteria include lactic acid bacteria, the main working horses of dairy industry, and Bacillus spp. that belong to the most occupied biocatalysts in global scale [22].

Fungi and yeasts

The fungi present in the investigated side streams include yeast S. cerevisiae and filamentous fungi Aspergillus and Trichoderma. S. cerevisiae is used in brewing and biofuel production for ethanol fermentation. Aspergillus spp. are used e.g. in production of foods, citric acid production and enzyme production. Trichoderma reesei is a common host for the production of industrial enzymes.

The cell wall of fungi determines the morphology and integrity of the organism during growth and cell division. The cell wall is formed by three groups of polysaccharides: polymers of mannose (mannoproteins), polymers of glucose (β-glucan), and polymers of N-acetylglucosamine (chitin), accounting for approximately 40%, 60% and 2% of the cell wall dry mass, respectively [23]. The structures of β-glucan and chitin are presented in Figure 3. The fungal cell wall is a dynamic structure that can adapt to physiological and morphological changes [24], and respond to environmental stresses by restructuring.

The basic structure of fungal cell wall consists of fibrillar β-1,3-glucan and chitin components [25] embedded to an amorphous matrix of mannoproteins. Chitin is mostly located near to the plasma membrane while the β-glucans are present throughout the cell wall [26]. Fungal cell wall contains ca. 10–15% chitin, while yeast cell walls contain only 1-2%. The structure, i.e. crystalline or amorphous forms, and deacetylation degree of chitin vary largely between fungal species, Ascomycota having the least acetylated chitin due to presence of glucans [27].

The matrix of yeast cell wall is composed most commonly of glycosylphosphatidylinositol proteins (GPI-CWP) which are linked to β-1,3- and β-1,6-glucans via glycosidic bond [28], or alkali-sensitive linked cell wall proteins (ASL-CWP). The cell wall matrix of filamentous fungi composes of galactomannoproteins and β-glucans. The inner layer of the fungal cell wall is electron-transparent and

galactose. e water-soluble biosorbent was precipitated by ethanol from metal solution a er reaching the equilibrium, which may not be feasible in actual applications [76,77].

In comparative biosorption study for Pb removal by Çolak et al. [78] two heavy metal resistant bacteria Bacillus strains, B. pumilus and B. cereus

Source or form, pretreatment					

research and the weights of their probable impacts on biosorption applications are summarized in the Table 6.

In the present research the majority of the produced side streams originated from yeast fermentations. Principally, S. cerevisiae has bene cial properties regarding biosorption applications. It is however notable that the availability of the material relays essentially from the interests of the side stream producer which should be motivated to ensure a steady supply of the side stream throughout the year [88]. As the yeast residues are currently sold in open market for feed manufacturers, more economically attractive alternative would be

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