Changes in Bacterial Community Structure in Wastewaters in the presence of *Saccharomyces Cerevisiae* and Benzalkonium Chloride

Changes in Bacterial Community Structure in Wastewaters in the presence of *Saccharomyces Cerevisiae* and Benzalkonium Chloride

L Žorža[1*], M Kalniņš[1], D Gudrā[2], K Megnis[2], D Fridmanis[2], A Rapoport[1], O Muter[1]

[1]Institute of Microbiology & Biotechnology, University of Latvia, 1 Jelgavas Str., Riga LV-1004, Latvia.
[2]Latvian Biomedical Research and Study Centre, 1 Ratsupites Str, Riga LV-1067, Latvia.

*Email: laura.zorza@lu.lv

Abstract. The benefit of bioaugmentation of activated sludge (AS) with yeasts was tested using AS and wastewaters (WW) that originated from the chemical industry, as well as synthetic WW. A stimulating effect of Saccharomyces cerevisiae on microbial respiration intensity in AS was shown. The added yeast biomass maintained viability in AS, an inhibitory effect of BAC on *Bacilli* was found; however, the addition of yeasts to AS reduced this effect.

1. Introduction

Yeasts play an important role in biotechnological processes. They are increasingly used for the production of various foods, alcoholic beverages, enzymes, pharmaceutical proteins and other valuable products [1,2] Due to the widespread use of yeasts in industry, the problem of spent yeast biomass (SYB) is related to the reduction of wastes by implementing the principles of a circular economy.. Recently, various environmental laws have been adopted to reduce waste and maximise the use of products in factories. For example, brewers are interested in minimising product losses from fermentation and minimising waste streams. Brewing spent yeast is the second major byproduct of the brewing industry with a considerable environmental impact due to the disposal of a large quantity of biomass (1 hL of beer generates 2.0-4.0 kg of SYB) [3,4].

Yeast biomass contains proteins, ribonucleic acid, lipids, ash and fibre in concentrations up to 49, 6, 3, 9 and 24%, respectively [5]. Due to the high nutritional value of yeast, it is often used as an animal feed [2,6]. Additionally, yeast can be used as a resource for processing high added value products for human consumption, e.g., β-glucans, dietary supplements, antioxidants, antimicrobials etc. [4,7,8,9].

Another yeast application field is related to environmental biotechnologies, particularly wastewater (WW) treatment. Yeasts can be used for the degradation of xenobiotics and the sorption of heavy metals [10,11,12]. Yeasts have been found in activated sludge (AS) [13,14,15], although AS is mainly dominated by heterotrophic bacteria and eukaryotic organisms such as protozoa and rotifers [16]. Yang et al. (2011) studied the distribution and diversity of yeasts in three typical full-scale plants processing biopharmaceutical, papermaking and municipal WW [17]. Yeasts from 21 different genera were found, mainly *Rhodotorula, Candida, Trichosporon, Pichia* and others [17]. The yeast of *S.cerevisiae*, along with *Pseudomonas* were

dominant in the AS after 185 days of continuous operation in the aerobic treatment of winery WW [18]. In turn, our recent study on a microbial community structure of the sewage sludge has revealed a negligible abundance of fungal DNA, particularly 0.01% reads [8].

Yang et al. have shown that the nutrient composition and dissolved oxygen levels in WW greatly influence yeast concentration and diversity in WW, which in turn impact the treatment process [17]. Physiologically active yeasts can be involved in breakdown of organic compounds, including xenobiotics [19, 20]. The addition of SYB to coal industry wastewater effluents promotes the biodegradation processes, thereby increasing CH4 production intensity and reducing COD (chemical oxygen demand) in anaerobic membrane bioreactor [21]. In addition, yeast biomass can serve as a source of nutrients (e.g., carbohydrates, proteins, free amino acids, ash, vitamins and fatty acids) for AS microorganisms, thus contributing to the efficiency of the purification process [4,22]. In our study, the preliminary testing of industrial AS has not revealed any abundance of culturable yeasts. Therefore, it was hypothesised that the addition of yeast biomass to industrial AS could notably change the bacterial community structure, bacterial physiological state and response towards xenobiotics. Benzalkonium chloride (BAC) was chosen as a model contaminant, which is used in the manufacture of various cosmetics, detergents and hand disinfectants and, therefore, is routinely discharged into WW and possesses the risk to the environment and human health [23, 24, 25,26]. Little is known about the potential impacts of BAC on AS and the performance of biological WW treatment processes.

The aim of this study was to evaluate the impact of *Saccharomyces cerevisiae* 14 on the composition of the AS bacterial community and its activity in the presence of BAC. Freshly cultivated yeast biomass was used rather than SYB in order to determine the potential of *S. cerevisiae* in this process. The methods of classical microbiology, Ion-Torrent PGM sequencing and OxiTop® microbial respiration tests were applied.

2. Materials and Methods

A culture of *S. cerevisiae* 14 was obtained from the collection of the Institute of Microbiology and Biotechnology, University of Latvia. The AS and WW were sampled at JSC BIOLARS. The WW had the following physico-chemical characteristics: chemical oxygen demand (COD) <1100 mg/L; biological oxygen demand (BOD5) 157 mg/L; Ntot <80 mg/L; Ptot <8 mg/L. The dry weight (dw) of AS in the samples was 2.8%. Samples were stored at 4°C and used for experiments not later than after 48 h of storage.

Synthetic WW had the following composition (mg/L): NaCl 1000.0; citric acid 50.0; ascorbic acid 30.0; sucrose 100.0; Na_2HPO_4 230.0 [27].

2.1. Experiment in the OxiTop® device

Effect of BAC on respiration intensity of AS amended with *S. cerevisiae*. Incubation of AS in WW and BAC was performed using the OxiTop® device (WTW, Germany). The OxiTop® biological oxygen demand (BOD) measurement system is based on the detection of pressure changes in a bottle containing a microbial culture due to the formation of CO_2 and its absorption by NaOH, which results in a decrease in pressure. Manometric measurement of pressure changes was performed automatically every 12 min. Data collected during the incubation were sent to the controller through an infrared interface and then to a computer using Achat OC software.

Incubation in the OxiTop® device was performed in three consequent stages with 1 h ventilating between stages, in order to supply the enclosed space with fresh air. In the first stage of the experiment, 50 mL AS was added to each 500 mL bottle and incubated for 48 h at 23°C in order to oxidise all bioavailable organics in the AS. Afterwards, 5 mL of a 20x stock solution of synthetic WW and 45 mL of sterile tap water were added to each bottle, resulting in dilution of AS up to 14 g dw/L. BAC was added to the bottles at the final concentrations of 10 and 30 mg/L in the second and third stages, respectively. A culture of *S. cerevisiae* was obtained after 48 h cultivation of on Yeast Extract-Peptone-Dextrose (YPD) agar at 30°C. The harvested

cells were suspended (10^8 CFU/mL) in a sterile saline solution (0.85% NaCl) and 1 mL of culture was added to the respective bottles. The incubation period of the stages 1, 2 and 3 was 48h, 96h and 96h, respectively. Two independent experiments were performed.

2.2. Enumeration of Culturable Microorganisms

For the enumeration of colony forming units (CFU) in AS, the following solidified media were used: 1) Standard Method Agar (SMA, BD, USA) for the total count; 1) Yeast Extract Peptone Dextrose Agar (YPD, LAB M, UK) to maintain yeast cultures and prepare the inocula; 3) Rose Bengal Agar Chloramphenicol (RBAC, Biolife, Italy) for the selective cultivation of yeasts; 4) Levine Eosin Methylene Blue Agar (EMD, BD, USA) for the enumeration of Gram-negative bacteria.

2.3. Ion Torrent PGM Sequencing

DNA was extracted using a FastDNA SPIN Kit for Soil (MP Biomedicals, USA). Polymerase chain reaction (PCR) of the 16S rRNA V3-4 region was performed employing Probio_Uni_F and Probio_Uni_R [28] primers tagged with 10-11 bp unique barcode labels along with the adapter sequence. PCR reaction was carried out using Phusion Hot Start II DNA Polymerase (Thermo Fisher Scientific, USA) and GeneAmp® PCR System 9700 (Thermo Fisher Scientific, USA) according to the manufacturer's guidelines. Thermal conditions of the PCR reaction were set as follows: 98°C for 30 seconds, 35 cycles of 98°C for 10 seconds, 67°C for 15 seconds, 72°C for 15 seconds with a final extension at 72°C for 7 minutes. PCR products were purified using NucleoMag® NGS Clean-Up and Size Select Kit (Macherey-Nagel, Germany). The quality and quantity of amplicons were assessed using the Agilent High Sensitivity DNA kit and an Agilent 2100 BioAnalyzer (Agilent Technologies, USA). Samples were diluted to 12 pM and pooled. Samples were sequenced on an Ion Torrent Personal Genome Machine sequencing platform employing Ion PGM™ Hi-Q™ View OT2 kit (Life Technologies, USA) for template generation and an Ion PGM™ Hi-Q™ View Sequencing kit (Life Technologies, USA) on an Ion 318 v2 chip (Life Technologies, USA). Each run was expected to produce approximately 300,000 reads per sample.

2.4. Mathematical Statistics and Data Analysis

Sequencing data analysis was carried out using QIIME version 1.8.0 and UPARSE pipeline version 7.0.1001 to quality-filter and cluster 16S rRNA amplicon sequences [29]. Sequences with the mean sequence quality score > 20 passed the quality control. Operational taxonomic units (OTUs) were built at 97% sequence identity with the UCLUST algorithm [30]. Taxonomic assignment to the lowest possible rank was performed with RDP [31], using the Greengenes [32] (http://greengenes.secondgenome.com) reference dataset (gg_otus-13_8 release). The alpha diversity metric Shannon index was calculated within the QIIME environment. Averages and standard deviations were determined using Microsoft Excel 2016. Significant differences between treatment types were assessed by single-factor ANOVA.

3. Results and Discussion

3.1. Microbial Respiration Intensity in the presence of 10 mg/L BAC

Incubation of AS was performed in the OxiTop® device in order to determine how the addition of yeast biomass and BAC influences the respiration intensity of microorganisms. The data presented in Figure 1 correspond to 24 h and 48 h of Stage 2, when the highest respiratory intensity was observed during the experiment. After 24 h of incubation, the respiration intensity varied in the range from 31.0 to 290.0 mg O_2/L, with the highest value found in the [AS-Y] set. Furthermore, [AS-Y] also had more intensive respiration in the presence of 10 mg/L BAC (AS-Y + BAC) compared to [AS], i.e. 87.4 ± 12.0 and 31.0 ± 4.0 mg O_2/L, respectively. However, after 96 h of incubation in variants without BAC, the respiratory

intensity in [AS] was higher than in [AS-Y], whereas the respiration intensity in the presence of BAC was higher in the set with yeast, i.e., [AS-Y+BAC] as compared to [AS+BAC] (Figure 1). Additional testing of microbial enzyme activity (i.e., fluorescein diacetate hydrolysis) in these samples showed a similar trend (data not shown).

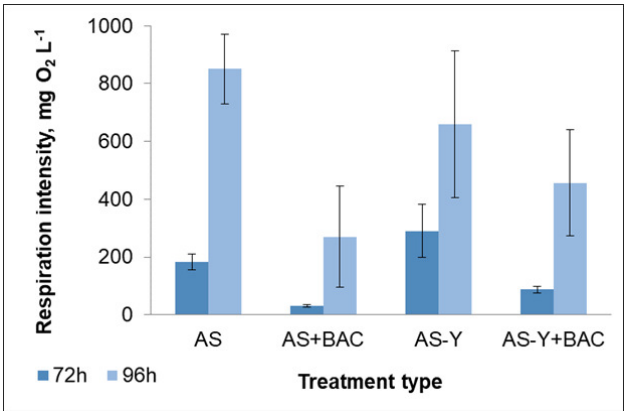

Figure 1. Respiration intensity of the activated sludge after incubation in the OxiTop® device.

Data correspond to 24 h and 48 h of incubation at the second stage. [AS] - activated sludge; [AC+BAC] - activated sludge + 10 mg/L benzalkonium chloride, added to AS at the 2nd stage; [AS-Y] - activated sludge + *S.cerevisiae* 14 (10^6 CFU/mL); [AD-Y+BAC] - activated sludge + *S.cerevisiae* 14 + 10 mg/L benzalkonium chloride, added to AS at the 2nd stage. Experiment setup as described in 2.1. Error bars represent the averages of two independent experiments ± SD, $p<0.05$.

The lowest respiratory intensity of [AS+BAC] after 96 h of incubation among the tested variants indicated that 10 mg/L BAC inhibited the physiological activity of microorganisms in the AS. Zhang et al. (2011) examined the effect of BAC on the respiratory intensity of heterotrophic microorganisms in the BAC concentration range from 5 to 70 mg/L [33]. It was shown that the specific oxygen uptake (SOUR) was reduced by half in the presence of 22 mg/L, compared to the reference culture [33]. So, the primary effect of BAC is considered to be based on the inhibition of respiratory enzyme activity in AS-derived microorganisms [33]. Our recent study with the yeast *Ogataea polymorpha* showed that the inhibitory effect of BAC on yeast respiration intensity depends on the carbon source used to obtain the yeast biomass through ethanol fermentation [34].

3.2. CFU count in Activated Sludge after Incubation in the presence of [10+30] mg/L BAC

After ten days of incubation with a gradual increase of BAC concentration from 0 mg/L to 30 mg/L, the CFU count did not reveal any inhibitory effect of BAC. As shown in Figure 2, after 10 day incubation, the total number of aerobic heterotrophic bacteria and Gram- negative bacteria in [AS] was similar and reached $4.6*10^5$ CFU/mL, while the presence of BAC in [AS+BAC] resulted in an increased CFU count up by one order of magnitude. Moreover, the addition of yeast cells to AS stimulated the growth of CFUs in both EMB and SMA media, reaching $(6.3±0.7)*10^7$ and $(4.4±0.5)*10^7$ in [AS-Y]; $(1.0±0.6)*10^8$ and $(1.2±0.7)*10^8$ in [AS-Y+BAC], respectively (Figure 2). The number of yeast CFUs after 10 days of incubation was not notably changed compared with the initial biomass of *S. cerevisiae* added to AS, and was found to be $(1.0±0.3)*10^5$ and $(7.0±1.0)*10^5$ in [AS-Y] and [AS-Y+BAC], respectively (Figure 2).

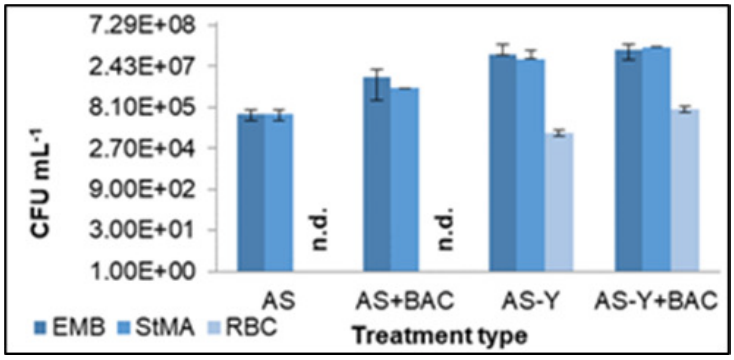

Figure 2. The number of culturable microorganisms in activated sludge after three cycles of incubation performed in the OxiTop® device (10 days incubation at 23°C).
[AS] - activated sludge;
[AC+BAC] - activated sludge + 10 mg/L and 30 mg/L benzalkonium chloride, added to AS at the 2nd and 3rd stage, respectively;
[AS-Y] - activated sludge + S.cerevisiae 14 (10^6 CFU/mL);
[AD-Y+BAC] - activated sludge + S.cerevisiae 14 + 10 mg/L and 30 mg/L benzalkonium chloride, added to AS at the 2nd and 3rd stage, respectively. N.d. – not detected. StMA - Standard Methods Agar; EMB - Levine Eosin Methylene Blue Agar; RBC - Rose Bengal Agar with Chloramphenicol. Experiment setup as described in 2.1. Error bars as in Figure 1.

The bacteriostatic and bactericidal effects of BAC have been reported to be species- specific and dependent on environmental conditions. As was reported by Bridier et al. (2011), the minimum bactericidal concentrations (MBC), i.e. reducing the initial population by five log10 units, for *Staphylococcus aureus* and *Enterococcus faecalis* were 70 mg/L and 52 mg/L BAC, respectively. These bacterial species exhibited the highest resistance to BAC among the 77 bacterial strains tested. For other bacterial species in WW, a lower MBC was found, e.g., 16 mg/L and 24 mg/L for *Pseudomonas aeruginosa* and *Escherichia coli*, respectively [1]. Moen et al. (2012) reported on the survival of a small (1-5%) subpopulation of *Escherichia coli*, which maintained tolerance to BAC after serial transfers in medium without BAC [35].

There are several different mechanisms (both BAC-specific and general stress responses) responsible for the regrowth of a tolerant subpopulation in the presence of BAC [36]. In our study, the BAC concentration [10 + 30] mg/L was likely to have a negative effect on the physiological processes of the microorganisms but was not high enough to be bactericidal.

3.3.Bacterial Community Diversity in AS after Incubation in the presence of 0 mg/L BAC, 10 mg/L BAC and 30 mg/L BAC

Taking into consideration the notable changes in a microbial metabolic activity in AS subjected to BAC and bioaugmentation with yeast biomass (Figures 1 and 2), it was expected that some specific shifts in the structure of AS bacterial community may occur. Incubation of AS in the OxiTop® resulted in increasing the bacterial community's Shannon diversity index, i.e. from 7.72 in the control (non-incubated) sample up to 8.72-9.36 after incubation. This effect may be explained by the addition of nutrients to AS, as the synthetic WWs contain citric acid, ascorbic acid and sucrose (50 mg/L, 30 mg/L and 100 mg/L, respectively (see Materials and methods) [15]. At the phylum level, two dominant taxa, i.e. Firmicutes and Proteobacteria, were found in the AS, with their abundance in the control (non-incubated AS) and all incubated AS samples being in the range of 27-35% and 22-36%, respectively (Figure 3A). These bacteria are known to be degraders of different xenobiotics. Particularly, in the BAC-contaminated AS, *Pseudomonas* and

Achromobacter were identified as the most abundant bacteria with a high biodegradation potential, with an integrase and a dioxygenase involved in BAC biotransformation [30]. The abundance of Actinobacteria and Bacteroidetes representatives slightly decreased as a result of incubation compared to the non-incubated AS, but no considerable changes in the bacterial community structure at the phylum level were observed (Figure 3A). At the class level, incubation of AS resulted in an increased abundance of Clostridia, i.e., from 4% in the non-incubated AS up to 18-25% after incubation. Conversely, the proportion of Bacilli was decreased from 29% in the non- incubated AS to 3-14% after incubation. Yeast biomass stimulated the abundance of Gammaproteobacteria, reaching 11% and 15% in [AS-Y] and non-incubated AS, respectively. However, this effect was reduced in the presence of 10 mg/L and 30 mg/L BAC to 11% and 9%, respectively (Figure 3B). In contrast, the addition of BAC to the AS slightly stimulated the survival of Betaproteobacteria during incubation, regardless of the presence of yeast, as well as Alphaproteobacteria in AS without yeast (Figure 3B).

Changes in the bacterial community structure at the genus level indicated a stimulatory effect of the added yeast on *Alcaligenaceae*, *Carnobacteriaceae* and *Fusibacter* spp. abundance in the presence of BAC. In contrast, in the yeast-free variants, increased levels of the OTUs of *Clostridiales*, *Rhodobacteraceae* and *Alcaligenaceae* were observed in the presence of BAC (Figure 3C). It should be noted that in mapping hierarchical data communities at the genus level, representatives of some genera remain identified only at the family level (Figure 3C).

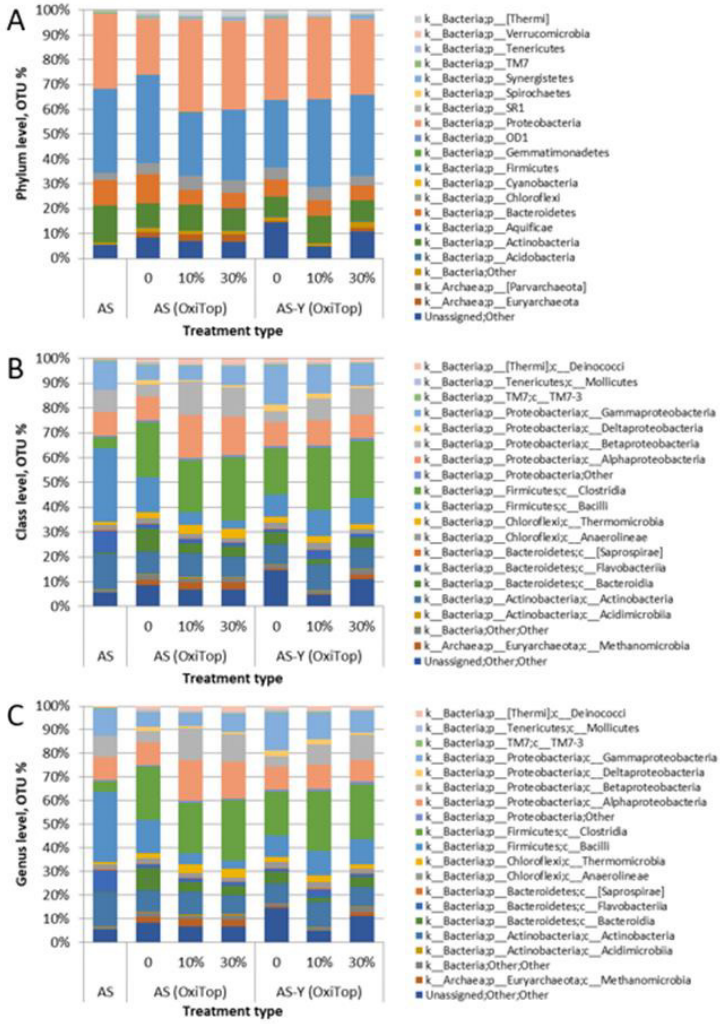

Figure 3. Activated sludge bacterial community composition at the phylum (A), class (B) and genus (C) levels after 10 day incubation in the OxiTop® device in synthetic wastewaters amended with *S. cerevisiae* 14 and/or BAC. Experiment setup as described in 2.1. [AS] - activated sludge; [Y] - *S.cerevisiae* 14 (10^6 CFU/mL). Concentration of BAC 0%, 10% and 30 %. The values <1% are not indicated in the diagram.

4. Conclusions

Results indicated that bioaugmentation of WW with yeast biomass considerably influenced the bacterial community structure and microbial metabolic activity of contaminated WW. First, the culturable yeast cells survived in WW in the presence of BAC and without it, after 10 days of incubation. Furthermore, bioaugmentation stimulated the growth of bacterial CFUs in both EMB and SMA media. Second, addition of yeast biomass resulted in an increased respiration intensity of microorganisms in WW. Particularly, after 24 h of incubating AS in synthetic WW, the microbial respiration intensity in the [AS-Y] set was 58% higher than in [AS], while in [AS-Y+BAC] set - 182% higher than in [AS+BAC]. Third, considerable changes in the bacterial community structure in AS after incubation with *S. cerevisiae* 14 and BAC were demonstrated.

The inhibitory effect of BAC on *Bacilli* was reduced in the presence of yeast. The addition of yeast to AS stimulated the abundance of *Alcaligenaceae*, *Carnobacteriaceae* and *Fusibacter* spp. in [AS-Y+BAC]. All these findings confirm the possible benefits of WW bioaugmentation with yeast biomass in relation to the WW treatment process, especially in the context of the removal of antimicrobial agents. However, mechanisms of these processes are still poorly understood. Further studies will be focused on physico-chemical changes in WW during treatment.

Acknowledgements
Study was financially supported by the European Regional Development Fund (ERDF) project 1.1.1.1/16/A/113 "Development of new approaches for waste-less simultaneous production of bioethanol, furfural and other valuable products from local agricultural waste material". Authors are grateful to JSC Olaine chemical plant BIOLAR for the samples and useful discussions.

References
[1] Rai AK, Pandey A, Sahoo D 2019 Biotechnological potential of yeasts in functional food industry. *Trends Food Sci. Technol.*
[2] Liu Y, Espinosa CD, Abelilla JJ, et al 2018 Non-antibiotic feed additives in diets for pigs: A review. *Anim. Nutr.*
[3] Hellborg L, Piškur J 2008 Yeast diversity in the brewing industry. In: Beer in Health and Disease Prevention
[4] Fărcaş AC, Socaci SA, Mudura E, et al 2017 Exploitation of Brewing Industry Wastes to Produce Functional Ingredients. In: *Brewing Technology*
[5] Victral DM, Aquino SF, Silva SQ, Baêta BEL 2016 Application of residual yeast as a source of redox mediators for the anaerobic decolorization of a model azo dye. *Brazilian J Chem Eng.* https://doi.org/10.1590/0104-6632.20160334s20150270
[6] Djuragic O, Levic J, Sredanovic S 2009 Evaluation of homogeneity in feed by method of microtracers. *Archiva Zootechnica* **12(4)**, 85-91.
[7] Podpora B, Świderski F, Sadowska A, et al 2016 Spent brewer's yeast extracts as a new component of functional food. *Czech J Food Sci.* https://doi.org/10.17221/419/2015-cjfs
[8] Vieira EF, Carvalho J, Pinto E, et al 2016 Nutritive value, antioxidant activity and phenolic compounds profile of brewer's spent yeast extract. *J Food Compos Anal.* https://doi.org/10.1016/j.jfca.2016.07.006
[9] Kerby, C. VF 2017 An Overview of the Utilisation of Brewery By-Products as Generated by British Craft Breweries. Beverages 3. https://doi.org/doi:10.3390/beverages3020024
[10] Zhang L, Liu N, Ma X, Jiang L 2013 The transcriptional control machinery as well as the cell wall integrity and its regulation are involved in the detoxification of the organic solvent dimethyl sulfoxide in Saccharomyces cerevisiae. FEMS Yeast Res. https://doi.org/10.1111/1567-1364.12022
[11] Yang M, Zheng S 2014 Pollutant removal-oriented yeast biomass production from high-organic-strength industrial wastewater: A review. *Biomass and Bioenergy*
[12] Wang T, Zheng X, Wang X, et al 2017 Different biosorption mechanisms of Uranium(VI) by live and heat-killed Saccharomyces cerevisiae under environmentally relevant conditions. *J Environ Radioact.* https://doi.org/10.1016/j.jenvrad.2016.11.018
[13] Shchegolkova NM, Krasnov GS, Belova AA, et al 2016 Microbial community structure of activated sludge in treatment plants with different wastewater compositions. *Front Microbiol.* https://doi.org/10.3389/fmicb.2016.00090
[14] Third KA, Paxman J, Schmid M, et al 2005 Enrichment of anammox from activated sludge and its application in the CANON process. *Microb Ecol.* https://doi.org/10.1007/s00248-004-0186-4

[15] Evans TN, Seviour RJ 2012 Estimating Biodiversity of Fungi in Activated Sludge Communities Using Culture-Independent Methods. *Microb Ecol.* https://doi.org/10.1007/s00248-011-9984-7
[16] Fakhru'l-Razi A, Zahangir Alam M, Idris A, et al 2002 Filamentous fungi in Indah Water Konsortium (IWK) sewage treatment plant for biological treatment of domestic wastewater sludge. *J Environ Sci Heal - Part A Toxic/Hazardous Subst Environ Eng.* https://doi.org/10.1081/ESE-120002830
[17] Yang Q, Angly FE, Wang Z, Zhang H 2011 Wastewater treatment systems harbor specific and diverse yeast communities. *Biochem Eng J.* https://doi.org/10.1016/j.bej.2011.09.012
[18] Petruccioli M, Cardoso Duarte J, Eusebio A, Federici F 2002 Aerobic treatment of winery wastewater using a jet-loop activated sludge reactor. *Process Biochem.* https://doi.org/10.1016/S0032-9592(01)00280-1
[19] Kaszycki P, Koloczek H 2002 Biodegradation of formaldehyde and its derivatives in industrial wastewater with methylotrophic yeast Hansenula polymorpha and with the yeast-bioaugmented activated sludge. *Biodegradation.* https://doi.org/10.1023/A:1020423517235
[20] Hashem M, Alamri SA, Al-Zomyh SSAA, Alrumman SA 2018 Biodegradation and detoxification of aliphatic and aromatic hydrocarbons by new yeast strains. *Ecotoxicol Environ Saf.* https://doi.org/10.1016/j.ecoenv.2017.12.064
[21] Yun YM, Kim M, Kim H, et al 2019 Increased biodegradability of low-grade coal wastewater in anaerobic membrane bioreactor by adding yeast wastes. *J Environ Manage.* https://doi.org/10.1016/j.jenvman.2018.12.083
[22] Dionisi D, Bertin L, Bornoroni L, et al 2006 Removal of organic xenobiotics in activated sludges under aerobic conditions and anaerobic digestion of the adsorbed species. *J Chem Technol Biotechnol.* https://doi.org/10.1002/jctb.1561
[23] Ertekin E, Hatt JK, Konstantinidis KT, Tezel U 2016 Similar Microbial Consortia and Genes Are Involved in the Biodegradation of Benzalkonium Chlorides in Different Environments. *Environ Sci Technol.* https://doi.org/10.1021/acs.est.5b05959
[24] Zhang C, Cui F, Zeng G ming, et al 2015 Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment. *Sci. Total Environ.*
[25] Wessels S, Ingmer H 2013 Modes of action of three disinfectant active substances: A review. *Regul Toxicol Pharmacol.* https://doi.org/10.1016/j.yrtph.2013.09.006
[26] Lei X, Teng-Fei S, Ce-Hui M, et al 2016 Related Environmental Problems and Research Progresses of Quaternary Ammonium Compounds (QACs). *Prog Chem.* https://doi.org/10.7536/PC151129
[27] Liu H, Chen J, Wu N, et al 2019 Oxidative degradation of chlorpyrifos using ferrate(VI): Kinetics and reaction mechanism. *Ecotoxicol Environ Saf.* https://doi.org/10.1016/j.ecoenv.2018.11.132
[28] Milani C, Hevia A, Foroni E, et al 2013 Assessing the Fecal Microbiota: An Optimized Ion Torrent 16S rRNA Gene-Based Analysis Protocol. *PLoS One.* https://doi.org/10.1371/journal.pone.0068739
[29] Pylro VS, Roesch LFW, Morais DK, et al 2014 Data analysis for 16S microbial profiling from different benchtop sequencing platforms. *J Microbiol Methods.* https://doi.org/10.1016/j.mimet.2014.08.018
[30] Edgar RC 2010 Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* https://doi.org/10.1093/bioinformatics/btq461
[31] Wang Q, Garrity GM, Tiedje JM, Cole JR 2007 Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol.* https://doi.org/10.1128/AEM.00062-07
[32] DeSantis TZ, Hugenholtz P, Larsen N, et al 2006 Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol.* https://doi.org/10.1128/AEM.03006-05

[33] Zhang M, O'Donoghue MM, Ito T, et al 2011 Prevalence of antiseptic-resistance genes in Staphylococcus aureus and coagulase-negative staphylococci colonizing nurses and the general population in Hong Kong. *J Hosp Infect.* https://doi.org/10.1016/j.jhin.2011.02.018
[34] Muter O, Khroustalyova G, Rimkus A, et al 2019 Evaluation of the enhanced resistance of Ogataea (Hansenula) polymorpha to benzalkonium chloride as a resource for bioremediation technologies. *Process Biochem.* https://doi.org/10.1016/j.procbio.2019.08.026
[35] Bridier A, Dubois-Brissonnet F, Greub G, et al 2011 Dynamics of the action of biocides in Pseudomonas aeruginosa biofilms. *Antimicrob Agents Chemother.* https://doi.org/10.1128/AAC.01760-10
[36] Moen B, Rudi K, Bore E, Langsrud S 2012 Subminimal inhibitory concentrations of the disinfectant benzalkonium chloride select for a tolerant subpopulation of Escherichia coli with inheritable characteristics. *Int J Mol Sci.* https://doi.org/10.3390/ijms13044101