Pollutant removal-oriented yeast biomass production from high-organic-strength industrial wastewater: A review

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ABSTRACT

Microbial single-cell-protein (SCP) production from high-organic-strength industrial wastewaters is considered an attractive method for both wastewater purification and resource utilization. In the last two decades, pollutant removal-oriented yeast SCP production processes, i.e., yeast treatment processes, have attracted a great deal of attention from a variety of research groups worldwide. Different from conventional SCP production processes, yeast treatment processes are characterized by higher pollutant removal rates, lower production costs, highly adaptive yeast isolates from nature, no excess nutrient supplements, and are performed under non-sterile conditions. Furthermore, yeast treatment processes are similar to bacteria-dominated conventional activated sludge processes, which offer more choices for yeast SCP production and industrial wastewater treatment. This review discusses why highly adaptive yeast species isolated from nature are used in the yeast treatment process rather than commercial SCP producers. It also describes the application of yeast treatment processes for treating high-carbohydrate, oil-rich and high-salinity industrial wastewater, focusing primarily on high-strength biodegradable organic substances, which usually account for the major fraction of biochemical oxygen demand. Also discussed is the biodegradation of xenobiotics, such as color (including dye and pigment) and toxic substances (including phenols, chlorophenols, polycyclic aromatic hydrocarbons, etc.), present in industrial wastewater. Based on molecular information of yeast community structures and their regulation in yeast treatment systems, we also discuss how to maintain efficient yeast species in yeast biomass and how to control bacterial and mold proliferation in yeast treatment systems.

1. Introduction

Yeasts are a group of unicellular fungi widely distributed in nature, most of which belong to two separate phyla: the Ascomycota and the Basidiomycota. Yeasts have played
important roles in the fermentation and food industries for thousands of years. Their obligatory acidophilic properties suggest that the fungi would not act as an opportunistic pathogen [1]. The protein extracted from yeast biomass, single cell protein (SCP), can replace costly conventional proteinaceous sources (e.g., soymeal and fishmeal) for animal feeds.

High-organic-strength industrial wastewaters often represent a significant loss of resources and causes serious pollution problems [2]. SCP production from these wastewaters is an attractive approach to both wastewater purification and resource utilization [3]. In most cases, the analyzed SCP composition mainly includes protein concentration, amino acid profiles, vitamins, carbohydrates, fats, and nucleic acids [4]. Since World War II, many yeast species have been used to produce SCPs from industrial wastewaters, a process that has been very important for numerous chronically malnourished people worldwide [5]. However, the conventional SCP process requires a pure yeast strain, expensive sterilization processes [6], optimized culture conditions (e.g., pH adjustment, extra nutrient supplement [N, P, Mg, Ca, Fe, Zn, Cu, Mn, and vitamins] [7], dilution rates [8]), and an air saturation of ≥20% dissolved oxygen (DO) by aeration [9] to achieve the maximum yeast biomass production. This results in high SCP production costs, low organic removals, and high nutrient residues that require post-treatment to control their discharge.

After the 1980s, numerous highly adaptive yeast strains for various industrial wastewaters have been isolated from a variety of sources (e.g., wastewater-contaminated soil, activated sludge) to replace commercial SCP yeast species due to their higher pollutant removal performances [10]. The pollutant removal-oriented SCP production processes, i.e., yeast treatment process, is characterized by higher pollutant removals, lower production costs, isolated yeast species, no excess nutrient supplements, and is performed under non-sterile conditions in a system similar to bacteria-dominated conventional activated sludge process (ASP) [6,11,12]. Its organic loading amounts to at least 15 kg·m⁻³·d⁻¹ chemical oxygen demand (COD) [13], which is nearly 10 times higher than conventional ASPs, thus offering more choices for yeast SCP production and industrial wastewater treatment.

2. Yeast species used in yeast treatment process

For conventional SCP processes, higher SCP production and protein content, and more plentiful and balanced amino acids essential for animal feed tend to make the selected yeast species or strain more attractive [14]. The most popular SCP yeast species are from the genera Candida, Hansenula, Pichia, Torulopsis and Saccharomyces [4]. In many cases, Candida utilis is frequently used for biomass production from a variety of carbon sources due to its high SCP production and specific growth rate [14,15]. However, the yeast species found in industrial wastewater treatment systems represent 48 taxa belonging to 21 different genera, of which the most frequent populations are from the genera Rhodotorula, Candida, Trichosporon, Pichia and some unidentified Ascomycetes [16]. This suggests that those commercial SCP yeast species may not be the most suitable for yeast treatment processes. For example, Candida langeronii appeared superior to C. utilis for biomass production from hemicellulose hydrolysate since the latter cannot utilize L-arabinose and grow at 42 °C [14].

Yeast strains of differing origins have different pollutant removal potentials [17], and isolated yeast strains obtained by spontaneous selection pressure in wastewater often reduce more COD and produce more yeast biomass than conventional SCP producers [10,18]. Furthermore, specific yeast species often selectively utilize preferential carbon sources prior to other carbon sources [14], or use metabolic byproducts generated by other yeast species in mixed culture [2]. In other words, mixed yeast cultures often result in higher biomass yield and greater pollutant removal from industrial wastewater containing a variety of carbon sources [2,11]. Therefore, yeast treatment processes use highly adaptive mixed yeast isolates rather than commercial SCP producers to treat corresponding industrial wastewaters.

3. High-organic-strength wastewater treatment

Yeast strains show high tolerance to low pH, high salinity, high-content organics, antibiotics, and sterilizers [19], and can metabolize various carbon substrates, including sugars (e.g., glucose, sucrose and maltose), biopolymers (e.g., starch, cellulose, hemicellulose and pectin), pentoses, methanol, alcohols, polyols, hydrocarbons, fatty acids and organic acids [7]. Industrial wastewaters are often highly acidic (pH < 5) and require a pH adjustment to 6.5-7.6 to reduce pH toxicity against bacteria for ASPs or anaerobic processes [20]. Following the yeast treatment process, the pH levels of these acidic industrial wastewaters often rise to neutral [6,10,21], which reduces wastewater treatment costs and facilitates subsequent ASPs. Therefore, yeast treatment processes appear to be more suitable and cost-effective for the treatment of acidic, oily, high-salinity, ammonia- or sulfate-ridden high-organic-strength industrial wastewaters that are not suitable for direct treatment by anaerobic processes. In addition to soluble organic substances, most yeast directly assimilate ammonium ions, urea, inorganic phosphates and sulphates [7]. However, higher nitrogen removal for the yeast system compared to the bacterial system has been attributed to higher nutrient (nitrogen and phosphorous) uptake in the yeast cells compared to bacterial cells [22]. In recent years, an in vitro detectable polyphosphate-synthesizing activity has been characterized in extracts of the yeast Candida humicola, and its properties were similar to those of a range of bacterial polyphosphate kinase enzymes [23].

3.1. High-carbohydrate wastewater

Yeast can metabolize various carbon substrates; however, they mainly utilize sugars (e.g., glucose, sucrose and maltose) [7]. Therefore, the concept of using yeasts to bioconvert high-carbohydrate wastewaters has long attracted the attention of SCP researchers. High-carbohydrate wastewater for SCP processes is produced widely in many food processing industries [13], e.g., starch processing wastewater [24], waste cassava starch hydrolysate [9], deproteinized whey [25], and defatted
Yeast SCP production from high-carbohydrate wastewaters discharged from vegetable processing industries. Recent studies have examined different vegetable processing wastewaters for yeast biomass production (Table 1), e.g., water extracts of cabbage and watermelon [26,27], pineapple cannery effluent [8,28], silage effluent [10], sugar cane bagasse hydrolysate [14], waste capsicum powder [15], and bamboo wastewater [29]. For example, a filamentous yeast strain (Galactomyces geotrichum) was isolated from silage effluent, and its ability to grow on and purify silage effluent was assessed in comparison with a strain of C. utilis. The yeast isolate finally achieved 91–95% COD removals and gave consistently higher biomass yields than C. utilis, producing a maximum of ~9 g·L⁻¹ [10]. Furthermore, wastewaters from various fermentation processes, e.g., pharmaceutical, alcoholic [30], butanol [31], vitamin, glutamate [6] industry, etc., also contained sugar residues as the main COD constituent. An isolated yeast, identified as Pichia anomala, achieved maximum COD reduction (81%) from ergot alkaloid production wastewater [18]. The binary yeast isolates Candida halophila and Rhodotorula glutinis achieved 85% COD reduction and 96% sugar reduction from glutamate fermentation wastewater [32].

3.2. Oil-rich wastewater

Considerable quantities of oil-containing wastewater are released from the food processing industries (e.g., slaughterhouse, dairy, and oil manufacturing) and tanneries. For example, olive mill wastewater (OMW) treatment has become a critical environmental challenge for the Mediterranean countries, which account for approximately 95% of the world olive production [17]. In these edible oil manufacturing plants, a considerable amount of fatty acid in raw vegetable oil is separated from glyceride through a washing process and then discarded as oil-rich industrial wastewater [33]. Dairy wastewaters often contain high levels of fats and esters (i.e., essentially triglycerides consisting of straight-chain fatty acids attached to glycerol) and their hydrolytic products (i.e., long chain fatty acids) [34]. A wide spectrum of different types of yeasts, e.g., Candida tropicalis, Candida rugosa, Candida lipolytica and Saccharomyces lipolytica, are able to grow on lipids [35]. In Japan, nine strains of yeasts capable of decomposing oil were isolated to directly treat soybean oil manufacturing wastewater (10 000 mg·L⁻¹ oil) with no pretreatment and to achieve an effluent with 100 mg·L⁻¹ oil in an 1-year pilot operation [11]. In comparison with other yeast isolates (Rhodotorula rubra, Candida boidini, Trichosporon cutaneum), C. utilis had the greatest oil uptake rate and highest specific growth rate [3]. Ten isolated yeast strains stably removed >89% COD and >99% oil from vegetable oil-containing wastewater (15 g·L⁻¹ COD and 10 g·L⁻¹ oil) [36].

3.3. High-salinity wastewater

Vegetable, tanning, fermentation and seafood processing industries often generate large quantities of saline high-organic-strength wastewater. The majority of bacterial species tend to dehydrate and disintegrate due to the high osmotic pressure differences between the protoplasm and the ambient high saline conditions, which severely inhibits growth and performance of bacterial systems [22]. It is reported that application of salt-tolerant bacteria (e.g. Halobacter halobium) in ASPs resulted in better treatment performances at salt contents above 2% [37]. As an alternative to salt-tolerant bacteria, osmotolerant yeast culture was more efficient for aerobic treatment of high salinity wastewater compared to bacterial culture [22]. For example, the osmotolerant yeast, Pichia guilliermondii, was selected from 70 yeast isolates to produce SCP from soy-rich waste brine from a kimchi factory containing 10% NaCl, and removed approximately 90% biochemical oxygen demand (BOD) within 24 h [38]. Six strains of yeast, Saccharomyces sp., Pichia sp., Rhodotorula sp., Candida sp., Kluyveromyces sp. and Trichospora sp., grew well in lettuce brine from the vegetable fermentation industry under aerobic conditions [21]. The binary yeast isolates C. halophila and Rhodotorula glutinis achieved a constant COD removal of >80% from the wastewater (salinity 12%) during 2-months of continuous operation of a fixed-bed reactor [6]. The mechanisms underlying the adaptation of various yeasts to salt stress involve the accumulation of osmotically active compounds (mainly glycerol) to counterbalance an increased external osmotic pressure and the modification of plasma membrane transport systems to extrude Na⁺ from the cell [39].

4. Xenobiotic biodegradation by yeast

Besides high-strength biodegradable organic substances, which usually contribute the major fraction of the BOD, many

<table>
<thead>
<tr>
<th>Wastewater origins</th>
<th>Countries</th>
<th>Reducing sugar (g·L⁻¹)</th>
<th>Yeast species</th>
<th>Biomass production (g·L⁻¹)</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste Chinese cabbage [26]</td>
<td>South Korea</td>
<td>18.5</td>
<td>Candida utilis, Pichia stipitis, Kluyveromyces marxianus, Saccharomyces cerevisiae</td>
<td>6–10</td>
<td>35–44</td>
</tr>
<tr>
<td>Cabbage, watermelon, green salads, and tropical fruits [27]</td>
<td>Singapore</td>
<td>1.4–8.9⁴</td>
<td>Saccharomyces cerevisiae</td>
<td>6–8</td>
<td>40–45</td>
</tr>
<tr>
<td>Pineapple cannery waste [8]</td>
<td>India</td>
<td>7.5</td>
<td>Candida utilis</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Silage effluent [10]</td>
<td>UK</td>
<td>40.5⁵</td>
<td>Galactomyces geotrichum</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Bamboo wastes [29]</td>
<td>China</td>
<td>~40</td>
<td>Candida utilis</td>
<td>19</td>
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⁴ The concentration of dissolved organic matter (g·L⁻¹).
⁵ COD concentration (g·L⁻¹).
industrial wastewaters contain xenobiotics, such as color substances (including dye and pigment) and toxic xenobiotics (including phenols, chlorophenols, antibiotics, polycyclic aromatic hydrocarbons (PAHs), etc.). While dyes are commonly used in food and textile dyeing/finishing industries, other color substances are also produced by various industrial processes, for example melanoidin pigment, a dark brown pigment found in the molasses wastewater [40]. The presence of very small amounts of color substances in wastewater (less than 1 mg-L^{-1} for some dyes) is highly visible and affects the aesthetic merit, water transparency and gas solubility. Furthermore, the OMW are very phytotoxic and possess strong antimicrobial activities principally due to phenolic compounds, such as tyrosol and hydroxyrosol [17]. Avermectin fermentation wastewater contains toxic avermectins, macrolytic lactones as pesticides and antiparasitic drugs that inhibit anaerobic bacteria [19]. Methods for BOD removal from these industrial wastewaters are fairly well established; however, toxic xenobiotics are generally resistant to microbial degradation due to their synthetic origin and complex aromatic molecular structures [40]. Therefore, the removal of these xenobiotics from wastewaters is often more important than the removal of soluble biodegradable organic substances. A variety of yeast species are known to secrete enzymes capable of degrading xenobiotic compounds. The proliferation of yeast cells in industrial wastewater is often accompanied by a concomitant xenobiotic biodegradation [41], e.g., color substances [40,42–45], phenol- and chlorophenol-related substances [46–50], PAHs [51,52], and other toxic xenobiotics [19,41].

The final yeast SCP product should not only be nutritious, but toxins, hydrocarbons, petroleum or heavy metals accumulated during the course of growth should be removed for the SCP to pass all toxicity tests and to be commercialized as a food product [4,53]. Since some high-organic-strength industrial wastewaters can contain xenobiotics, and the yeast biomass from yeast treatment process contains bacterial cells, necessary toxicity measurements (e.g., feed experiments [54]) should be considered to assure the suitability of the resulting yeast SCP for animal feed. Additionally, the yeast SCP from some fermentation wastewater containing toxic fermentation products (e.g., antibiotics) can be reused as raw material or a nitrogen source for commercial fermentation of identical fermentation products to avoid the side effect of those toxic residues.

4.1. Color substances

Although several yeasts may remove dyes through biosorption mechanisms [42], there have been numerous reports on decolorization by yeast through biodegradation. For example, two yeast isolates, Debaryomyces polymorphus and C. tropicalis, may produce manganese dependant peroxidase and decolorize six azo dyes and one antraquinone dye by cometabolism [43]. Among the 2402 strains of yeast isolates from various sources, a strain identified as Issatchenka orientalis showed the highest potential for decolorization of anaerobic-treated molasses wastewater with melanoidin pigment, COD and BOD removals of 91%, 80% and 77%, respectively, at 30 °C and pH 5.0 during a 7 day batch culture [40]. Subsequently, this strain provided a constant decolorization yield about 70% during 3 replacement cycles [40]. The yeast Candida zeylanoides degraded a number of simple azo dyes with color removals ranging from 44% to 90% [44]. The Kluyveromyces marxianus IMB3 decolorized Remazol Black-B dye with the maximum color removal of 98% at 37 °C and pH 3.0–5.5 due to biosorption to the yeast cells rather than a metabolic reaction [45].

4.2. Phenol- and chlorophenol-related substances

Two wild type strains of Yarrowia lipolytica could grow in OMW with 19 g-L^{-1} of COD and approximately 800 mg-L^{-1} of total phenols, leading to 80% COD degradation and 70% total phenol reduction [46]. A fluidized bed reactor loaded with C. tropicalis immobilized onto granular activated carbon was capable of efficiently removing phenol as the sole carbon source at volumetric loading rates as high as 60 mg-L^{-1}·h^{-1} phenol [47]. A total of 32 cold-adapted, psychrophilic and cold-tolerant yeast isolates from alpine habitats, including Cryptococcus terreus, Cryptococcus terricola, Rhodosporidium lusitaniae, Rhodotorula creatinivora, Rhodotorula ingeniosa, Mastiogasidium intermedium, and Sporobolomyces roseus, were able to degrade phenol and 18 phenol-related mono-aromatic compounds at low temperatures [48]. A pure culture of C. tropicalis isolates could degrade 2000 mg-L^{-1} phenol and 280 mg-L^{-1} m-cresol within 66 and 52 h, respectively, and the presence of m-cresol significantly inhibited phenol biodegradation while a low phenol concentration accelerated the assimilation of m-cresol [49]. Among the most investigated yeast strains capable of degrading phenol and chlorophenol (C. tropicalis, Candida maltosa; Trichosporon oivide, Trichosporon cutaneum, and Rhodotorula glutinis), C. tropicalis is a hydrocarbonoclastic yeast capable of metabolizing phenol, resorcinol, quinol, hydroxyquinol, catechol, and to a lesser extent protocatechuate, p-cresol, m-chlorophenol, and p-chlorophenol via the b-ketoacidipate pathway by an inducible-enzyme system [50].

4.3. PAHs

Pichia anomala, isolated from soil contaminated with crude oil, degraded four PAHs (naphthalene, dibenzothiophene, phenanthrene and chrysene) both alone and in combination [51]. Five PAHs-degrading yeast isolates from the oil-contaminated soil in Jidong Oilfield in China, including C. maltosa-like, Pichia guilliermondii, Rhodotorula dairenensis, Sporidiobolus salmonicolor and Pichia anomala, efficiently degraded ~99% of low molecular weight PAHs and ~89% of high molecular weight PAHs at room temperature within 6 weeks [52].

4.4. Other toxic xenobiotics

The methylotrophic yeast Hansenula polymorpha can utilize formaldehyde at concentrations up to 1750 mg-L^{-1}, levels toxic to most microorganisms, for treating methanol and formaldehyde-containing chemical industry wastewater through methylotrophic pathway reactions [41]. C. tropicalis, which was screened from avermectin fermentation wastewater and showed tolerance to avermectins residue, removed 67% COD and 99% avermectins [19].
5. Biocontrol of yeast treatment system

Molecular fingerprinting techniques, including polymerase chain reaction-based denaturing gradient gel electrophoresis [55], cloning and sequencing of rRNA genes [56,57], and fluorescent in situ hybridization-flow cytometry [20,56,58], allow for accurate identification, quantification and community structure analyses of the microbial population. The rapid development of these methods in the past two decades provides new insight into the microbial community structure and thus favored biocontrol in yeast treatment systems.

A large loss of yeast species and even biomass washout is often observed during the transformation from batch culture to a continuous culture [11,12], which influences treatment performance in some cases [12]. For example, of the five isolated yeast species Rhodotorula rubra, C. tropicalis, C. utilis, C. boidinii and Trichosporon cutaneum, only C. tropicalis ultimately remained in the aeration tank, possibly due to its optimal settleability [12]. Among the ten yeast species isolated from soil contaminated by wastewater, only C. lipolytica, C. tropicalis, and C. halophila were dominant in the system, which was determined by the surface hydrophobicity and emulsification ability of yeast cells rather than COD removals, biomass yield, cell settleability and cell flocculation ability [36]. Therefore, the application of efficient yeast isolates does not imply their presence and action inside a wastewater treatment system. Furthermore, the internal relationships between the operational parameters and yeast community structure are of considerable importance for the treatment performance. Several clone library analyses revealed that DO level (aerobic and microaerobic) was a critical factor affecting the yeast community structure and treatment performance in yeast treatment systems [56,57]. The aerobic DO level (>2 mg·L⁻¹) resulted in more determined yeast species, poor biomass settling, and higher COD removals, while the microaerobic DO level (<0.5 mg·L⁻¹) effectively restored the microbial biomass settlement, as well as produced unacceptable COD removals and fewer determined yeast species [56]. The influent COD level and hydraulic retention time (HRT), two primary components of the influent COD loading rate, have different effects on the structure and function of the yeast community when a higher influent COD loading rate is achieved [55]. Therefore, a higher influent COD level at identical HRT supported more yeast species and thus the metabolism of a greater variety of carbon sources, while a shorter HRT at identical influent COD level supported fewer yeast species and thus the metabolism of fewer carbon sources [55].

Because yeast treatment systems are generally open systems, the substrate also promotes the growth of bacterial or mold species present in the mixed liquor when the yeast species propagate continuously. The propagation of a specific yeast strain in the yeast treatment system is based on free competition among different microorganisms [22]. Therefore, the major challenge for yeast treatment processes is to properly design and control operational conditions to guarantee a yeast-dominated biomass, as well as good biomass settling under non-stereile conditions. Because sterilization for conventional SCP process is expensive, the fact that yeast can grow at low pH can be used to: (i) Sterilize the water at relatively low temperatures, (ii) prohibit contaminating bacteria from growing, and (iii) provide sufficient environmental selection pressure in a continuous system to direct a complex microflora towards yeast domination [35]. Therefore, an acidic pH (<6) is generally used for yeast treatment processes to maximize yeast growth and limit bacterial growth under the non-sterile conditions [11,20,55,59]. However, based on fluorescent in situ hybridization-flow cytometry analyses, Zheng et al. [20] found that it was difficult to achieve yeast-dominated biomass in continuous-flow systems at low COD loadings (e.g., 2 kg·m⁻³·d⁻¹ COD), regardless of the acidic pH levels, while acidic pH was sufficient to achieve yeast-dominated biomass in a batch culture. In other words, the yeast treatment process should be conducted with both acidic pH and high COD loading (e.g., 8.7 and 21.0 kg·m⁻³·d⁻¹ COD) levels as prerequisites [20]. If yeast cells are larger than bacterial cells, producing a lower surface-to-volume ratio, lower substrate adsorption/uptake efficiency at low substrate concentrations or loading may result.

Although bacteria have difficulty competing with yeast at acidic pH, molds do not encounter this problem [35]. However, most molds are extremely sensitive to CO₂ and even 10% CO₂ is sufficient to stop their growth in a competitive situation [35]. Furthermore, many yeast species, including Candida fennica, Candida pelliculosa, Candida silvicultrix, Pichia anomala, Pichia burtonii, Pichia farinosa and Pichia membranifaciens have validated their mold biocontrol ability, i.e., strongly inhibiting inoculated and endogenous molds (e.g., Penicillium roqueforti and gray mold (Botrytis cinerea)) [60]. In fact, few reports are available on mold growth and dominance in yeast treatment systems. Many clone library analyses revealed that all clonal fungal sequences inside yeast treatment systems were of yeast rather than mold origin [56]. In previous studies, the mold Geotrichum candidum was suspected to successfully propagate in yeast treatment systems and result in poor settling and deteriorated treatment performance [33]. However, subsequent investigations have demonstrated that these filamentous fungal species obtained in yeast biomass could be specific yeast species with two different cell morphologies (conidia and hyphae) [56].

6. Conclusion

The yeast treatment process uses highly adaptive mixed yeast isolates under non-sterile conditions rather than commercial SCP producers under sterile conditions, which achieves higher pollutant removals and lower SCP production costs. The process appears to be cost-effective for the removal of high-strength biodegradable organic substances from acidic, oily, high-salinity, ammonia- or sulfate-ridden high-organic-strength industrial wastewaters that are not suitable for direct treatment by anaerobic processes. The proliferation of yeast cells in these industrial wastewater is often accompanied by a concomitant xenobiotic biodegradation including color substances and toxic xenobiotics. In the ASP-like open system, the internal relationships between the operational parameters and yeast community structure are of considerable importance for the successful application of efficient yeast isolates. Furthermore, in continuous-flow yeast treatment
systems, operating at both acidic pH and high COD loading levels inhibits the growth of bacterial species present in the mixed liquor while few molds grow and dominate in yeast biomass possibly due to the presence of CO₂ and/or some yeast species with mold biocontrol ability.

Acknowledgment

This study was supported by the Natural Science Foundation of China (51378066 and 21077011) and the New Century Excellent Talents in University (NECT-11-0044).

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