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**Journal of Brewing and Distilling (JBD)** provides rapid publication (monthly) of articles in all areas of the subject such as Fermentation Technology and Product Analysis, health effects of gin, Filtration and Packaging, Malt induced Premature Yeast Flocculation etc.

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# **Journal of Brewing and Distilling**

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## **Journal of Brewing and Distilling**

## Review

# Solid wastes in brewing process: A review

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A large amount of agro-industrial waste is produced annually around the world from the beneficiated agricultural products or in food industrialization. The disposal of these residues in the environment results in a lot of inconvenience to the ecosystem, due to its significant nutritional value and high concentration of organic compounds that confers a high biochemical oxygen demand to the waste's degradation. In this context, brewing industry is among these activities, which includes in its production stages the processing and fermentation of vegetable feedstock, such as barley malt and other grains, and hops, generating several by-products. Many factors, such as environmental policies, possible scarcity of non-renewable sources, and problems related to the improper use of renewable raw materials, leads to the development of new processes that could generate less waste or reused those produced in order to add greater value to the residue. This article presents a review of the solid wastes in brewing industry, which are the brewer spent grain, the hot trub, the residual yeast and the diatomaceous earth, describing how they are obtained in the brewery process, their characterization and chemical composition, and the potential applications in bioprocesses technologies. The main fraction common to all revised waste is the protein fraction, in addition to various constituents of interest, such as minerals, carbohydrates and phenolic compounds. The main current applications are in the area of animal feed and human nutrition.

Key words: brewery wastes, brewer spent grain, trub, residual yeast, diatomaceous earth.

#### INTRODUCTION

Beer is a millennial alcoholic beverage that allows consumers to taste different types and styles depending on how the production process is conducted and/or raw materials which are used. Overall, beer is a yeast fermenting product of the brewer wort obtained from malted cereal (barley), supplemented or not with other

cereals or sources of sugars, called adjuncts, with the addition of hops (Tschope, 2001; Rehm and Reed, 1983; Prescott and Dunn, 1949).

For many centuries, beer production on artisanal scale was sufficient to attend the demand, producing different varieties of good quality beers. However, the diffusion

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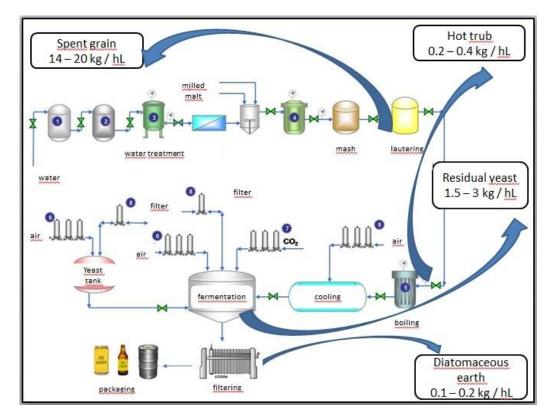


Figure 1. The brewery flow chart and the waste generation. (Adapted from www.3m.com).

and acceptance of this beverage throughout its history made it one of the most appreciated and consumed beverage in the world, in many countries with different climates and cultures. Thus, with the advent of the Industrial Revolution, beer production became a large scale process to meet the high demand. This factor characterizes the beer market until today, which develops modern technologies to attend the growing consumer demand.

Several technological advances in the last 20 years have provided the brewing industry large savings by lowering generation of by-products in the process. However, certain wastes inherent to beverage production hardly have their quantities formed reduced, such as brewer spent grains, residual brewing yeast and trub, due to the necessity of grain processing, the characteristics of chemical composition and treatment of the raw materials used, and the need for microbial activity during fermentation (Priest and Stewart, 2006), respectively.

These three residues, called wet brewery wastes, are responsible for the loss of approximately 20 L per 100 L of water used in the brewing process, especially because of the high water content of those, between 80 and 90%. This promoted great drag of wort and loss of extract, as well as of beer, depending on which step the residue is generated, leading to significant amounts of effluent

formation (Priest and Stewart, 2006).

A fourth brewery residue may be also mentioned: diatomaceous earth, used in the filtration of final product to improve its brightness. The production of this waste can be avoided by using other filter media, or even commercializing unfiltered beer, naturally cloudy, typically consumed in the form of special or handmade beers. However, in large scale breweries and countries where it is a habit of consumption of clear beer, this filter element is the most used.

These waste generated in the process presents a high content of organic substances and, therefore, a wide variety of potential applications in feed, food and industrial biotechnology. In this review article, are discussed the four industrial byproducts related to the brewing process, its generation, the amounts generated and its potential biotechnological applications.

# GENERATION, CHEMICAL COMPOSITION AND POTENTIAL APPLICATIONS OF THE WASTES

The generation of these wastes in their respective stages of the brewing process as well as the medium amounts formed are detailed throughout the work and can be summarized as shown in Figure 1.

ParameterSpent grainHot trubResidual yeastDiatomaceous earth slurryFibers $\sqrt{\phantom{a}}$ --Carbohydrates- $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$ Protein $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$ Free aminoacids $\sqrt{\phantom{a}}$ - $\sqrt{\phantom{a}}$ Ash $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$ Vitamins $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$ Phenolic compounds $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$ Fatty acids- $\sqrt{\phantom{a}}$  $\sqrt{\phantom{a}}$ 

**Table 1.** Main components presents in the brewery wastes\*.

Brazil, has the largest percentage of shares of AB InBev, the major brewery industry in the world, and is the third major in the production ranking (12.4 billion L/year), just after the United States (22.5 billion L/year) and China (48.9 billion L/year) (Cervesia, 2011). According to the average amount of waste formed in the process (discussed below), the total production of these three world producers generates about 16.9 million tons/year of spent grain, 250 thousand tons/year of hot trub, 2.1 million tons/year brewery residual yeast and 348 thousand tons/year of diatomaceous earth (values calculated from the average of the literature data).

Fossil materials

These waste shows significant and rich chemical composition, whose main components of the four brewery wastes that are investigated in this review are summarized in the Table 1.

In order to obtain products with higher added value and destine these waste to nobler purposes, industrial bioprocesses present themselves as potential application (Pandey et al., 2000). Regarding beer industry, spent grain, hot trub and residual brewery yeast can be highlighted, mainly due to their rich composition, of high nutritional value organic compounds, as well as their minerals content. Also, as residues they could be used as low cost feedstock in formulation of wort in many industrial fermentation processes.

Below are discussed the four industrial byproducts related to the brewery process, the importance of volume and potential biotechnological applications.

## Brewer spent grain

The brewer spent grain, or malt bagasse, is the first solid waste to be generated throughout the process, taking into account the modern model of breweries, which purchase the malt from malting industries. During the mashing step, there is an exhaustion of malted grains milled as all the important soluble compounds which constitute the sweet wort are extracted. In this step the bagasse formed has an important role as a filter element. This residue has a high nutritional value and it is the largest solid residue

produced, resulting in a great volume of residue throughout the year with low or no cost to its acquisition (Aliyu and Bala, 2011).

This residue corresponds to about 85% of the total waste generated in the brewing process (Lima, 2010). Normally, for every 100 kg of processed grains, 125 to 130 kg of wet bagasse are generated, containing about 80 to 85% of moisture as obtained in filtration tanks, or 50 to 55%, when obtained in press filter. This amount corresponds to around 14 to 20 kg of bagasse for each hectolitre of beer produced (Fillaudeau et al., 2006; Reinold, 1997), which draws between 0.5 and 1% of the produced wort extract (Priest and Stewart, 2006).

During the mashing process, about 80% of the malt mass is solubilized, remaining in the bagasse the insoluble fractions. Although its composition varies with the species of barley, the malting process, as well as the malt processes of milling, mashing and clarification (Celus et al., 2006; Santos et al., 2003), exhausted malt are predominantly a fibrous material with significant protein content, containing nutritional value equivalent to about one fifth of the value of barley (Lima, 2010; De-Song et al., 2009). The bagasse content can be between 15 to 26.2% of proteins and 70% of fibers, those which can be divided in three fractions; cellulose (between 15.5) and 25%), hemicelluloses (mainly arabinoxylans, 28 to 35%) and lignin (approximately 28%). It may also contain lipids (between 3.9 and 10%), ash (2.5 to 4.5%), vitamins, amino acids and phenolic compounds (Aliyu and Bala, 2011; Lima, 2010; Robertson et al., 2010; Mussato et al., 2006).

Among the mineral components, are calcium, phosphorus, and selenium. It also contains biotin, choline, folic acid, niacin, pantothenic acid, riboflavin, thiamine and vitamin B6. Among the amino acids are leucine, valine, alanine, serine, glycine, tyrosine, lysine, proline, threonine, arginine, cystine, histidine, isoleucine, methionine, phenylalanine, tryptophan, glutamic and aspartic acids (Priest and Stewart, 2006).

The usual destiny to brewer spent grain is the commercialization to animal feed production, and it may also be

<sup>\*</sup>Compiled from several data from the literature, specified throughout the text.

**Table 2.** The potential application of brewer spent grain.

Animal feed and human nutrition  Energy and biogas production  Protein concentrates  Copi and Sang, 2013.  Niemi et al., 2013; Faulds et al., 2009; De-Song et al., 2009; Treimo et al., 2008; Markovic et al., 1995.  Obtaining of fermentation products as:  Ethanol  Lactic acid  Gums  Antibiotics  Enzymes  Cuptor for cell immobilization  Production of brewer wort for obtaining lowalcohol beer  Cultivation of microorganisms for single cell protein (SCP) production  Oil extraction  Energy and biogas production  Gopi and Sang, 2013.  Niemi et al., 2013; Faulds et al., 2009; De-Song et al., 2008; Markovic et al., 2008  Sencheva et al., 2012  Mussato et al., 2012  Mussato et al., 2008  Stredansky and Conti, 1999  Khan et al., 2009  Hashemi et al., 2011; Adeniram et al., 2010; Gregori et al., 2008.  Briggs et al., 2007; Plessas et al., 2007; Dragone et al., 2007.  Briggs et al., 2004.  Wang et al., 2001.  Priest and Stewart, 2006	Application	Reference			
Niemi et al., 2013; Faulds et al., 2009; De-Song et al., 2009; Treimo et al., 2008; Markovic et al., 1995.  Obtaining of fermentation products as:  Ethanol Lactic acid Gums Antibiotics Enzymes Support for cell immobilization Production of brewer wort for obtaining lowalcohol beer Cultivation of microorganisms for single cell protein (SCP) production  Niemi et al., 2013; Faulds et al., 2008; Markovic et al., 2009; Treimo et al., 2008; Markovic et al., 2012 Mussato et al., 2008 Stredansky and Conti, 1999 Khan et al., 2009 Hashemi et al., 2011; Adeniram et al., 2010; Gregori et al., 2008.  Kopsahelis et al., 2007; Plessas et al., 2007; Dragone et al., 2007.  Briggs et al., 2004.  Wang et al., 2001.	Animal feed and human nutrition				
Protein concentrates  Song et al., 2009; Treimo et al., 2008; Markovic et al., 1995.  Obtaining of fermentation products as:  Ethanol Lactic acid Gums Antibiotics Enzymes  Support for cell immobilization  Production of brewer wort for obtaining lowalcohol beer  Cultivation of microorganisms for single cell protein (SCP) production  Cencheva et al., 2012 Mussato et al., 2012 Mussato et al., 2008 Stredansky and Conti, 1999 Khan et al., 2009 Hashemi et al., 2011; Adeniram et al., 2010; Gregori et al., 2008.  Kopsahelis et al., 2007; Plessas et al., 2007; Dragone et al., 2007.  Briggs et al., 2004.  Wang et al., 2001.	Energy and biogas production	Gopi and Sang, 2013.			
Ethanol Lactic acid Gums Antibiotics Enzymes  Support for cell immobilization  Production of brewer wort for obtaining lowalcohol beer  Cultivation of microorganisms for single cell protein (SCP) production  Gencheva et al., 2012 Mussato et al., 2008 Stredansky and Conti, 1999 Khan et al., 2009 Hashemi et al., 2011; Adeniram et al., 2010; Gregori et al., 2008. Kopsahelis et al., 2007; Plessas et al., 2007; Dragone et al., 2007.  Briggs et al., 2004. Wang et al., 2001.	Protein concentrates	Song et al., 2009; Treimo et al., 2008;			
Lactic acid Gums Antibiotics Enzymes  Support for cell immobilization  Production of brewer wort for obtaining lowalcohol beer  Cultivation of microorganisms for single cell protein (SCP) production  Mussato et al., 2008  Stredansky and Conti, 1999  Khan et al., 2009  Hashemi et al., 2011; Adeniram et al., 2010; Gregori et al., 2008.  Kopsahelis et al., 2007; Plessas et al., 2007; Dragone et al., 2007.  Briggs et al., 2004.  Wang et al., 2001.	Obtaining of fermentation products as:				
Production of brewer wort for obtaining low-alcohol beer  Cultivation of microorganisms for single cell protein (SCP) production  Dragone et al., 2007.  Briggs et al., 2004.  Wang et al., 2001.	Lactic acid Gums Antibiotics	Mussato et al., 2008 Stredansky and Conti, 1999 Khan et al., 2009 Hashemi et al., 2011; Adeniram et al., 2010; Gregori et al., 2008.			
alcohol beer  Cultivation of microorganisms for single cell protein (SCP) production  Wang et al., 2004.  Wang et al., 2001.	Support for cell immobilization				
protein (SCP) production wang et al., 2001.	•	Briggs et al., 2004.			
Oil extraction Priest and Stewart 2006	o o	Wang et al., 2001.			
Thouast and Stoward 2000.	Oil extraction	Priest and Stewart, 2006.			

added to other process residues, for example, trub, brewery residual yeast, and diatomaceous earth (Briggs et al., 2004). Even though those are useful destinations, it can explored the use of this by-product in application in order to reach its rich nature in certain components.

According to Aliyu and Bala (2011), Lima (2010) and Mussato et al. (2006), many applications can be cited, such as animal and human nutrition, energy production by direct burning or for biogas production by anaerobic fermentation; charcoal production; adsorbent material in chemical treatments; cultivation of micro-organisms and obtaining bioproducts by fermentation; support for cell immobilization, among others. Table 2 summarizes the potential applications of the brewer spent grain

In a recent work, Vieira and collaborators (2014) studied the valuation of the brewer's spent grain proposing a recyclable integrated process for extraction of proteins and arabinoxylans in alkaline medium and its recovery by citric acid and ethanol addition, respectively; to be used as food ingredients.

Based on the high moisture content, nutritional value, and significant presence of residual fermentable sugars, the malt bagasse is very unstable and susceptible to microbial contamination, mainly by filamentous fungi. Therefore it should be promptly eliminated from the brewery. Thus, different conservation techniques may be proposed, which includes drying, freezing and addition of chemical preservatives. For any of those, usually the mass of bagasse is pressed to remove excess moisture, which should be slightly reduced to values close to 65%.

The extracted liquor returns to the process for the recovery of possible existing residual extract (Briggs et al., 2004).

Regarding the conservation methods used, drying is the most efficient one, since the high moisture content could easily cause contamination from microorganisms and increase weight (and volume) for storage and transportation. However, high energy cost makes the method less feasible (Aliyu and Bala, 2011). The main drying techniques are freeze drying and oven drying. Even though the first one does not promote changes in the material composition, it is not economically feasible. The hot drying at temperatures below 60°C is the most feasible method (Mussato et al., 2006).

Freezing techniques of wet material can also be used, with the disadvantage of the large volume generated since the high moisture content is not removed. Furthermore, the freezing temperature can promote alterations in sugar composition, for example, arabinose (Mussato et al., 2006). Chemical preservatives can also be added, such as ammonia and the lactic, acetic, formic, benzoic, propionic, and phosphoric acids, which is to maintain both, the quality and nutritional value of the by-product. Also be used as preservatives are potassium sorbate or common salt, NaCl (Lima, 2010; Priest and Stewart, 2006).

#### Hot trub

After the steps of mashing and clarification, the brewer

wort should be submitted to the boiling stage, with the purpose of hop addition and extraction of its aroma and bitterness compounds (isomerization); destruction of enzymes; colloidal stabilization; sterilization; dimethyl sulfide (DMS) and ketone compounds volatilization; development of color, flavor and aroma compounds; calcium phosphate precipitation with reduction of pH; concentration and adjusting the initial extract (Briggs et al., 2004; Bamforth, 2003; Kunze, 1999).

In this step, the wort, with high nitrogen content until this stage, loses part of this component (about 6%) due to the formation of a precipitate called hot trub. Hot trub is the second solid residue generated in the brewing process, which results predominantly from insoluble coagulation of mainly high molecular weight proteins. The molecules of those proteins tend to lose solvation water by heating, which promotes their denaturation. However, other substances may be present due to their participation in the formation of these complexes or due to drag during its deposition.

In addition to protein coagulation, the presence of cations, particularly Ca<sup>2+</sup>, may influence the neutralization of negative charges of proteins and peptides, promoting the formation of complexes. Hop compounds that have lower solubilization may also be precipitated. However, even the solubilized hop compounds may undergo electrostatic interaction with insoluble proteins, causing precipitating along with them. The hot trub may also contain minor amounts of low molecular weight proteins that have in their structure the specific amino acids proline, that can interact with polyphenols (mainly oxidized and condensed, especially tannins) and carbohydrates present in the medium (Priest and Stewart, 2006; Barchet, 1993). However, this last type of precipitate is more common in cold stages, comprising fermentation and maturation, since their interactions are unstable above 80°C temperatures (Briggs et al., 2004). In general, the average composition of hot trub may be described as (in dry matter): proteins (50-70%); hops bitter substances non isomerized (10-20%); polyphenols (5-10%); carbohydrates (4-8%), of which pectins, glucans and starch; minerals (3-5%); and fatty acids (1-2%) (Priest and Stewart, 2006; Barchet, 1993).

Several factors affect the trub formation process, its composition and quantity, such as: type of barley, composition, cultivation area, seasonal effects and malt drying process, proportion and types of adjuncts used, type of milling; profile and pH control of mashing process, medium concentration of ions and polyphenols, time, homogenization, pH (being 5.2 the optimum pH) and oxidation during boiling stage, primitive extract desired to the wort; type, concentration and degree of hops substances solubilization (Priest and Stewart, 2006). Coagulants and adsorbent can be added such as carrageenan gum to enhance trub formation (Barchet, 1993). In general, between 0.2 and 0.4 kg wet trub is

formed (80 to 90% of moisture) for each hectolitre of beer produced (Briggs et al., 2004).

The insoluble aggregate is formed of roughly spherical particles that tend to associate in flakes of approximately 10 cm in diameter, which precipitate on the medium dragging other wort components (Hough, 1990). Therefore, after boiling, wort should be clarified to remove precipitated material, since if it is not removed it may cause problems such as: deposition on subsequent pipelines and equipment; changes in pH; yeast coating (causes difficulty in nutrients assimilation); chelating effect (decreases ions availability for microbial activity); decreased colloidal stability of the final product due to previous rehydration of coagulated proteins; overload the final filtration; confer undesirable bitterness to beer; destabilization of the beer foam due to the presence lipid (Priest and Stewart, 2006). However, some authors claim the presence of trub can promote cell vitality and viability, as well as the fermentation process performance, due to the presence of lipids, minerals and protein sources (Bamforth, 2011; Kuhbeck et al., 2007).

Hot trub can be removed from the wort by filtration (with infusorial earth or perlite), by centrifugation, or by decantation tanks called whirlpool, which promotes centrifugal force and deposition of precipitated material in the center of the equipment (Barchet, 1993); effect known as tea cup effect. This last technique is the most commonly used in the industry. Whirlpool may be a distinct cylindrical tank to which the boiled wort is pumped tangentially into about 1/3 of the tank height, or it may be, the boiler itself, typically when the heat source is external and allows recirculation of the material by means of pumps. After application of centripetal force, a resting period is necessary to allow full sedimentation of particulates.

Trub removal promotes considerable losses of wort since its aqueous fraction is 80 to 90% and may represent extract reduction between 1 and 2% of the wort. Therefore, it is possible to recover part of the drawn wort by washing it with the next sweet wort, immediately after its production in the mashing process, or by centrifugation (Priest and Stewart, 2006). In general, trub is disposed over malt bagasse in clarification to be washed with the secondary water in order to recover part of the extract.

Commonly, the trub formed is mixed with the brewer spent grains or other ingredients for the preparation of animal feed (Priest and Stewart, 2006). However, its rich composition has significance potential for application in bioprocesses, aiming particularly towards exploitation of its protein concentration.

## Residual brewing yeast

In general, during the stage of fermentation, brewing yeast tends to multiply between 3 and 5 times in the

**Table 3.** Potential applications of residual brewing yeast.

Application	Reference
Animal feed and human nutrition	Man-Jin, 2005; Briggs et al., 2004.
Flavoring agentes production	Vieira et al., 2013a; Ferreira et al., 2010.
Filter elements for beverages clarification	Reinold, 2007.
Obtaining enzymes (invertase)	Hough, 1990.
Supplementation of maintenance and fermentation medias for micro-organisms	Ferreira et al., 2010; Jones and Ingledew, 1994.
Single cell protein (SCP) production	Chanda and Chakrabati, 1996.
Substrate for microalgae cultivation	Byung-Gon et al., 2013.
Biosorption and precipitation of heavy metals for remediation of soils and aqueous media	Chen and Wang, 2008; Marques et al., 2007; Marques et al., 1999; Ferraz and Teixeira, 1999; Butt, 1993.
Biogas production	Zupancic et al., 2012.

reactor, especially during the early hours, when oxygen is supplied to the wort (Briggs et al., 2004). Fermentation stage is followed by a resting period at low temperatures (maturation), when precipitation of the great yeast mass and other haze compounds occurs. The decanted yeast must be removed from the reactor to prevent autolysis, which promotes cytoplasmic material release as pH is raised, changing flavor, foam quality, microbiological stability, and resulting in darkening of the beer. Yeast removal is done by different drains in the bottom of the reactor during fermentation days.

It is common practice in brewing industry to reuse cell mass generated for inoculation of new fermentation tanks (Vieira et al., 2013b). The number of reuses depends on species, type of beer produced, content of the wort extract, and ensuring microbiological culture, and it may be between 3 and up to 10 times as long as it does not compromise the sensory quality of the beverage. Thus, as the possibility of cells recycling runs out, those cells must be removed from the process, generating new solid waste, which ranks second in production volume, called residual brewing yeast (Ferreira et al., 2010).

The amount of residual microbial biomass generated depends on the fermentation parameters (mainly aeration, temperature, and pH), type of microorganism (*S. uvarum* or *S. cerevisiae*), inoculum concentration, condition of the cell viability and vitality, as well as the composition of brewer wort. In general, the mass of yeast cells can be between 1.5 and 3 kg containing about 85 to 90% moisture, per 100 liters of beer produced (Olajire, 2012; Ferreira et al., 2010; Fillaudeau et al 2006, Blanpain-Avet and Daufin, 2006). Again, the large volumes of beer produced lead to the generation of significant quantities of waste, which has a high organic content (BOD) and needs adequate treatment for disposal, which means considerable costs (Briggs et al., 2004).

Residual brewing yeast is predominantly composed by proteins, ranging between 35 and 60% (dry basis), which

have high biological value (referring to the amount of essential amino acids in its structure), representing between 70 and 85% of casein value (Vilela et al., 2000a; Caballero-Cordoba et al., 1997). Amino acids present in greater quantity are lysine, leucine, isoleucine, valine, tryptophan, threonine, and phenylalanine, and there may be slight deficiency of sulfur amino acids (Yamada et al., 2003; Chae et al., 2000; Vilela et al., 2000b; Caballero-Cordoba and Sgarbieri, 2000; Sgarbieri et al., 1999).

In addition, this waste has other substances of importance to application, such as carbohydrates (35 to 45%), minerals (5 to 7.5%, of which, Ca, P, K, Mg, Fe, among others), lipids (4-6%), B vitamins, enzymes and RNA (Pinto et al., 2013; Bekatorou et al., 2006, Psarianos and Koutinas, 2006; Yamada et al., 2003).

The current major destination of brewery residual yeast is to formulate animal feed and to mix it with spent grain generated in the process to increase their nutritional value. Recently, new destinations have been explored, such as obtaining products with high nutritional value to the application in the pharmaceutical industry and in the human diet as dietary supplements due to their rich composition and to be generally recognized as safe (GRAS) (Man-Jin, 2005; Briggs et al., 2004). However, some limiting factors in their application to human consumption is the presence of bitter compounds, the difficulty to digest the thick cell wall and high RNA content, which can cause increase in the level of uric acid in the blood and tissues (Sgarbieri et al., 1999).

Different applications in the food science and technology, as well as various applications in industrial and environmental biotechnology can be seen in Table 3. The brewery residual yeast can be commercialized in paste form (as obtained after the fermentation process), powder (after dehydrated), or even in liquid form (after enzyme treatment, which increases its digestibility) (Tanguler and Erten, 2008). Some authors indicate that the process to obtain yeast extract increases protein concentration available in relation to intact cells (Yamada

et al., 2003; Caballero-Cordoba and Sgarbieri, 2000; Vilela et al., 2000a; Sgarbieri et al., 1999).

If yeasts are used intact, in general they must be inactivated by chemical or physical processes. Chemical agents, such as propionic and formic acids, or ethyl acetate can be used, which also as preservative. In thermal process for cell inactivation, research indicates that at 60°C membrane denaturation occurs, but not sufficiently to inactivate all internal enzymes, which occurs above 75°C. After dried, cells can be stored and, if for a long time, the addition of organic acids as preservatives may be required (Priest and Stewart, 2006).

In the case of yeast extracts preparation, cell lysis can be promoted by different methods, endogenous or exogenous, such as autolysis, hydrolysis and plasmolysis. Autolysis occurs by the natural action of endogenous enzymes when cells complete their growth cycle, reaching death stage. This process has some disadvantages such as low extraction yield, difficult solidliquid separation, unpleasant taste, and risk of deterioration caused by microbial contamination. In plasmolysis, the concentration of inorganic salts promotes cell lysis acceleration, although, it results in a product rich in undesirable salts. Hydrolysis is the most efficient method and it is done by acids or by proteolytic or cytolytic enzymes. Despite high performance, acid hydrolysis is not widely used due to the high initial investment cost and the possibility of formation of carcinogenic products such as mono and dichloropropanol (Chae et al., 2000).

In the processing for obtaining yeast extracts, it may be necessary to include a step to remove bitter substances from hops and trub, which tend to be adsorbed to the cell surface during beer fermentation (Shotipruk et al., 2005). This removal can take place by passing the residue on adsorption resins (polystyrene divinyl benzene), by microfiltration (Man-Jin et al., 2005) or alkaline washing (Pinto, 2011; Sgarbieri et al., 1999).

#### **Diatomaceous Earth**

The low-temperature fermentation and maturation as well as the low pH of beer promotes deposition of yeast and haze components in cold temperatures (Priest and Stewart, 2006). However, this is a slow process and it is not able to eliminate all medium turbidity (Lima et al., 2001). Additionally, there may be considerable turbidity and chemical changes in the beer flavor in its post-processing that is periods of transport and storage. These chemical changes occur mainly by oxidation, incidence of light, handling and temperature changes (Priest and Stewart, 2006; Kunze, 1999).

Thus, to obtain a stable and clear beer during the whole expiration period suggested, in general, clarifi-

cation techniques to remove turbidity materials (proteins, yeast, oxidized polyphenols,  $\alpha$  and  $\beta$ -glucans) should be used in order to promote greater colloidal stability and improve brightness and sensory quality to the beverage (Reinold, 2007; Markovic et al., 2003).

Filtration is the technique most widely used, combined with the addition of adsorbent agents. The process may take place in several steps until the desired clarity and transparency of the final product is achieved. Different filter element may be used in powder form, such as cellulose, diatomaceous earth, perlite, activated carbon, and even residual yeast from brewing itself. Press filter with cellulose fibers were widely used, however, their high operational cost lead to the use of diatomaceous earth filters or perlite (Reinold, 2007; Cancellara, 2004). Diatomaceous earth is a material rich in silicates from fossils of prehistoric algae (diatomites) (Hough, 1990). It presents a large surface area due to its excessive porosity, acting as an agent of depth filtration. It must be calcined to remove organic compounds and milled. Today, diatomaceous earth represents the most effective and used filtration method in brewing industry (Briggs et al., 2004).

A conventional filter of infusorian earth requires between 1 and 2 g of diatomaceous earth per liter of clarified beer. Due to the retention of organic material, especially yeast, proteins and polyphenols, by the end of the filtration, cake mass can be increased three times or more, and this material cannot be used in subsequent filtration after its saturation (Fillaudeau et al., 2006). Many recovering technologies to those filters have been developed such as chemical treatment or calcination in order to remove the organic matter and suspended solids to reopen the pores. However, such procedures are unable to regenerate the material completely, hindering its use in subsequent filtrations (Olaiire, 2012).

Thus, it generated another solid residue whose mineral composition depends on some factors: origin, formation time, and type of algae that was deposited over the years to form the diatomaceous earth. The organic composition, modified by the retention of particulate material present in beer, depends on the type of beer produced and treatments of raw materials and wort. From the organic material, the protein content can highlighted which can be 8 to 15% (w/w) (Russ et al., 2005).

Due to the high organic load and the large amount of suspended or dissolved material, the disposal of this waste in the environment is extremely difficult. Its disposal in the common sewer creates several difficulties to treat this effluent. Therefore, it can alternatively be disposed in landfills, which, however, can be a procedure of significant cost.

Also, reusing filtration residue represents considerable technical difficulties, mainly due to the high porosity of the material. The porosity retains organic material, making it required calcination for removal of those impurities.

Moreover, the high moisture content (around 70%) and chemical composition provide its rapid degradation, making their storage in ambient conditions difficult without previous treatment. If used as obtained (in the slurry form), small amounts of the residue may be mixed with soil as a source of organic matter, or added to the malt bagasse as animal feed for commercialization, although this last one has low acceptance (Briggs et al., 2004). If diatomaceous earth residue is treated by calcination it may be used to recover silicates, intended for construction applications (Russ et al., 2005).

## **FINAL CONSIDERATIONS**

The brewing process promotes the generation of three intrinsic waste, the spent grain, the hot trub and the residual yeast, whose amount generated cannot be reduced due to their stages of generation which is indispensable to the production process. Additionally, depending on the type of beer produced and the requirement to remove the turbidity of the beverage, there may be a filtration step, which promotes the generation of the fourth solid waste, the diatomaceous earth.

All residues studied in this review have high organic compounds content and significant fraction proteins as common fraction to them all. The spent grain has current application of greater relevance for animal feed and great potential for its use as a support for growth of immobilized microorganisms in industrial bioprocesses. The most relevant use of brewer's yeast is in animal feed and human nutrition, by obtaining products with high nutritional value.

Diatomaceous earth configures a large environmental disposal problem, since their use is complicated with the spent grain and destined for animal feed; their use in human nutrition is made difficult due to the high content of bitter hop compounds, requiring removal of these compounds in many because the high degree of porosity which retains its organic matter and its fossil composition. For all waste reviewed in this article, it can be observed that there is a potential for recovery of their protein fractions for obtaining products compatible with animal and human nutrition and for the development of microorganisms in industrial bioprocesses.

### **Conflict of Interest**

The author(s) have not declared any conflict of interest.

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