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CHAPTER
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Integration of treated agro-based wastewaters (TAWs) management with mushroom cultivation

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ABSTRACT

The inefficient management of wastewater generated from agro-based industries has become a cause of environmental degradation. Treated agro-based wastewaters (TAWs) are characterized by higher nutrients load and therefore, utilizable in the agriculture and horticulture as irrigation source. Agricultural reuse of TAWs is the most common practice done by the farmers of developing countries. Using freshwater for substrate wetting has been creating an extra load on our drinking water resources. Globally, a huge volume of freshwater is utilized for substrate formulation in the commercial mushroom cultivation and integration of TAWs with mushroom cultivation has presented improvements in the mushroom productivity, signifying their cultivation more profitable. Furthermore, spent substrates can be used for biogas production, animal feed for mature castrated male sheep, post-weaning calves feeding, biodiesel production, bioethanol production, reducing sugar production, biofertilizer production, methane production, butanol production, etc. This book chapter deals with sustainable approaches to the potential use of TAWs in the formulation of mushroom's substrate material along with efficient management of spent mushroom substrate.

KEYWORDS

Bioremediation, Industrial wastewaters, Mushroom cultivation, Water usage

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Introduction

Mushrooms are macro-fungi existing on the earth for 300 million years. As a foremost part of animal nutrition, they have been a good source of food and medicinal products (Valverde *et al*., 2015; Royse *et al*., 2017). Most of the edible and medicinal mushrooms can be grown on the selective type of agricultural wastes including, manure, sawdust, hardwood, soy hulls, wheat straw, rice straw, corn husk, peanut husk, wood lodges, mustard stem, crop leaves, and sugarcane bagasse, etc. (Grimm and Wösten, 2018). These substrates are known as bulk mushroom substrates and need further processing (nutrients addition) before application in the commercial cultivation. However, a substrate having specific and optimum contents of nitrogen, carbon, and minerals may be supportive for maximizing the growth and yield performance of mushroom as they can be easily broken assimilated by fungal mycelia (Bellettini *et al*., 2016). Therefore, substrate formulation is the first step in commercial mushroom cultivation which involves adjusting its nutrient content easily utilizable by mushrooms (Carrasco *et al*., 2018). For this, mushroom substrate/compost technology offers agricultural waste management through utilization as feedstock for large-scale mushroom production (Gyenge *et al*., 2016). Substrate formulation is achieved in a series of substantial steps such as substrate cutting, grinding, wetting, fertilizing, composting followed by pasteurization and sterilization. Sufficient water content in the substrate is essential for mushroom growth, therefore, freshwater is essentially used to wet the mushroom substrates commonly known as substrate wetting. It is estimated nearly 200 liters of fresh water are used to produce 1 Kg of white button mushroom (Udom *et al*., 2016). Using freshwater for substrate wetting has been creating an extra load on our drinking water resources, therefore, there is a new interest in developing more efficient substrate formulating technologies by using treated agro-based wastewaters (TAWs) as an alternative of freshwater (Kalmış *et al*., 2002; Kalmıs *et al*., 2008; Avni *et al*., 2017; Chang and Wasser, 2018).

Various agro-based industries such as sugar mill, palm mill, distillery, sago, oil-producing, dairy, food processing, molasses-based alcohol and beverages, tea and coffee, crop product processing, and biofertilizer producing industries are known to generate highly nutritive and less toxic wastewaters (Rebah *et al*., 2007; Rattan *et al*., 2015; Sadh *et al*., 2018). The treated agro-based wastewaters (TAWs) have been recognized for their irrigational application in horticulture and agriculture (Kretschmer *et al*., 2002; Shuval, 2012). The nutrient content accumulated in the agricultural soils after irrigating with such TAWs helps to enhance the growth and productivity of crop plants. It is estimated that nearly 20% of Mexico, 26% of Pakistan, 30% of India, 50% of Ghana, and 80% of Hanoi's agricultural land is being irrigated with both treated and untreated wastewaters generated from municipal and industrial sectors (Pedrero *et al*., 2010).

The idea of integrating TAWs with mushroom cultivation is not new but only a few reports

(Kalmıs *et al*., 2008; Avni *et al*., 2017; Chang and Wasser, 2018) are currently available focusing on this aspect, therefore, it requires more attention for scientific exploration. The views in previously published reports deal with either utilizing such as TAWs for wetting mushroom substrate or blending some agro-based solid wastes with the mushroom substrate. However, not all kinds of wastewaters can be used for wetting the substrates, but only a few TAWs are suitable to formulate the substrate materials of both edible and semi-edible mushroom species. Following the above aspects, this book chapter focused on the potential utilization of TAWs with agricultural wastes to grow mushrooms and further management of the spent substrate.

Waste decomposition and nutrients utilization by mushrooms

The main carbon and nitrogen sources required for the growth of mushroom comes from dead animal and plants (lignocellulosic) biomasses (Sánchez, 2009). Nevertheless, dead animal and lignocellulosic biomasses have never been exhausted on the earth assisting mushrooms to be one of the most primitive and successful organisms. The growth of a mushroom is divided into two phases including spawn running or mycelium growth followed by flush production or vegetative growth where actual biomass is produced (Montoya *et al*., 2012; Bellettini *et al*., 2016). During the mycelium growth phase, the fungal spores start propagating and spreading over the substrate surface creating a threadlike network system. This mycelium network covers the substrate surface and secrets a number of extracellular enzymes resulting in the degradation of lignin and cellulose (Table 1 and Figure 1).

Table 1. Mechanisms and enzymes involved in ligninolytic and non-ligninolytic substrate degradation by mushrooms.

Substrate type	**Enzyme(s) involved**	**Degradation mechanism(s)**
Ligninolytic	Phenoloxidases	Hydroxylation, free radical action, mediator
	Peroxidases	Production of quinones followed by ring fission
	Glucoseoxidases	Production of hydrogen peroxide
	Methyltransferase	Methylation of the carboxyl group
Non-ligninolytic	Aryl alcohol oxidase, Aldehyde reductase	Production of aldehydes and alcohols
	Cytochrome P450	Hydroxylation
	Cellobiose dehydrogenase	Fenton reaction

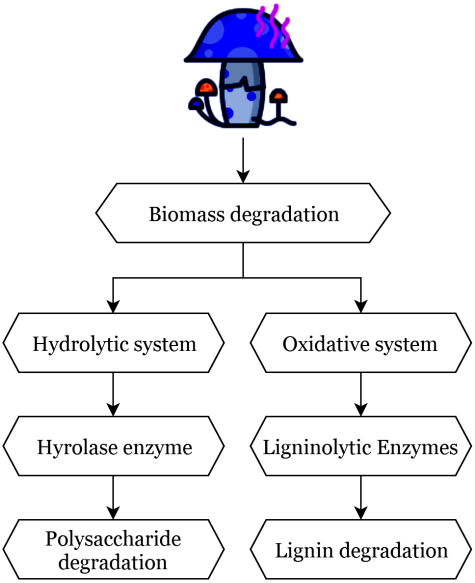

Figure 1. Mechanisms of lignocellulosic biomass degradation used by mushrooms.

The secretion of various intracellular and extracellular enzymes like peroxidases, ligninase, peroxidase, manganese, cellulases, pectinases, xylanases, and oxidases help them to breakdown the dead organic matter and convert them to lesser molecular and ionic forms which can be easily utilized by mushroom during its vegetative growth (Sánchez, 2009). Besides this, the process of breakdown is composite where multiple enzymes act to remediate a molecule from high molecular weight to low. These enzymes are induced by cytochrome P450 gene of fungi which have both ligninolytic and non-ligninolytic degradation capabilities (Kulshreshtha *et al*., 2014).

The nutrients, ions, and water contents are transported from substrate to mushroom body by means of both active and passive mechanisms (Smith, 1984; Randive, 2012). Specific physical and biochemical responses and stabilization for various nutrients in the mushroom are accomplished by myco-degradation, myco-stabilization, and myco-stimulation by mycelia, while bioaccumulation and myco-volatilization by the mushroom body (Chanda *et al*., 2016). However, certain abiotic and biotic factors such as temperature, humidity, relative substrate moisture, luminescence, atmospheric pressure, substrate pH, electrical conductance, available energy as carbon/nitrogen contents, minerals, interacting pests and pathogens, etc. may affect the nutrient transfer mechanism of mushrooms (Kulshreshtha *et al*., 2014).

There is an ultimate requirement of several micro and macronutrients for mushroom growth. In this regard, metal ions are transported to the mushroom body in the form of a metal-enzyme complex. The process involves two sequential steps *i.e.* ion regulation and ion uptake in which certain fungal genes such as Vps, Rbt, Ftr, Fet, Fre, Sit, Zrt, Pho84, etc. The genes help in secreting the extracellular enzymes which help to bind and transport them into the fungal cell wall. However, these genes may vary according to the mushroom species. Therefore, there is an essential requirement metal ion in mushroom growth and virulence (Gerwien *et al*., 2017). Besides this, the C/N content of the substrate strongly regulates the decomposition process by mushrooms. It has been reported in recent studies that the amendment of C/N rich substrates and fertilizers actively affects the rates of substrate breakdown and further nutrient uptake (Migliore *et al*., 2012; Kumar *et al*., 2019).

Nutrient values of treated agro-based wastewaters (TAWs)

Even after secondary treatment of wastewater from agro-based industries, a non-negligible amount of such utilizable nutrients is left. Despite, farmers prefer to use them as irrigation water due to their high nutrient values which results in rapid soil fertilization and high crop yields (Gothwal *et al*., 2012). It is found that long term soil irrigation using treated wastewaters having has shown excessive micronutrient accumulation, which affects microbial diversity and plant growth. However, mixing these TAWs with the mushroom substrate is a one-time practice, therefore, lesser will be the chances of excessive micronutrient accumulation. Below are the elementary nutrient values of TAWs which makes them useful for mushroom's substrate formulation.

Organic load: Organic content of TAWs is the total biodegradable dry biomass present in the suspended form (Kretschmer *et al*., 2002). The organic contents of TAWs come from the processed agro-based materials (organic compounds, complexes, microorganisms, residual biomass, etc.). Moreover, plant-based materials such as plant leaves, root, stem, fruits, juice, extracts, litter are also helpful to enrich TAWs with organic load (Kumar *et al*., 2018). The most common parameters of organic load are biological oxygen demand (BOD) and chemical oxygen demand (COD). Organic load of TAWs presents in both fixed and non-fixed forms which have been playing a crucial role in providing fertilizer values to the crops through wastewater irrigation. Microorganisms continuously feed and breakdown organic particles. As a result of redox reactions of microbial enzymes, these particles are converted into smaller and utilizable (by plant) molecular forms (Kwak *et al*., 2009). Therefore, the organic content of TAWs may be helpful in increasing the mushroom's substrate value, which may be helpful for increasing their growth and productivity.

Micronutrients: The elements required in trace quantity for life are known as micronutrients. Despite the proper functioning and metabolism of fungi, they perform an essential role in balanced mycelium growth. These elements include copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and molybdenum (Mo). Micronutrients are the foremost part of agro-based residues. TAWs contain sufficient micronutrients that can provide fertilization to the mushroom substrate. During the raw crop processing, these micronutrients are released in the wastewater. However, a significant amount of micronutrients is removed during the wastewater treatment process, therefore, TAWs are known to have a permissible level of micronutrients suitable for irrigation (Kumar *et al*., 2018). These micronutrients play a critical role in microbial growth and development.

Macronutrients: Macronutrients includes nitrogen (N), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and phosphorus (P). Macronutrients essentially play an important role in fungal growth and development. N play role in constructing the genetic material and protein synthesis while, Ca, Mg, K, and P have their own specified roles in various metabolic, growth, pathogen defense, and virulence (Gothwal *et al*., 2012). In TAWs, a significant quantity of these nutrient elements present. Higher amounts of macronutrients in discharged wastewaters may contribute essentially to fungal growth.

Other problems: Besides having the above utilizable contents, TAWs often come with numerous ion toxicity, heavy metals, microbial contamination, pesticide residues, and radioactive elements (Australia Standards, 2012). The fecal coliforms, fungi, bacteria, viruses, nematodes, protozoans are also the foremost part of discharged wastewaters.

Integration of treated agro-based wastewaters (TAWs) with mushroom cultivation

The integration of TAW with mushroom cultivation has given promising results. Agro-based substrates are supplemented with nutrients from different TAWs for enhanced mushroom productivity (Phan *et al*., 2012; Hanafi *et al*., 2018). However, there are only a few studies related to this, therefore, there is a strong need to explore the potential of certain TAWs in enhancing the nutrient values of mushroom substrates. The TAWs can be utilized as substrate moistening agents as an alternative to regular water supply (Figure 2 and Table 2). However, the substratum formulated by this method must be thoroughly sterilized before inoculated with mushroom spawn (BARC, 2018). Previously, certain experiments have been conducted to assess the effect of TAWs on mushroom productivity. Out of them, Wang *et al*. (2001) enhanced the efficiency of wheat straw by supplementing spent beer effluent for the cultivation of *Pleurotus ostreatus* mushroom. Olive mill wastewater was useful for the enrichment of wheat straw used to grow different *Pleurotus sp*. Kalmis and Sargin (2004). Distillery effluent was helpful for supplementation of wheat straw and bagasse used to grown three *Pleurotus* strains

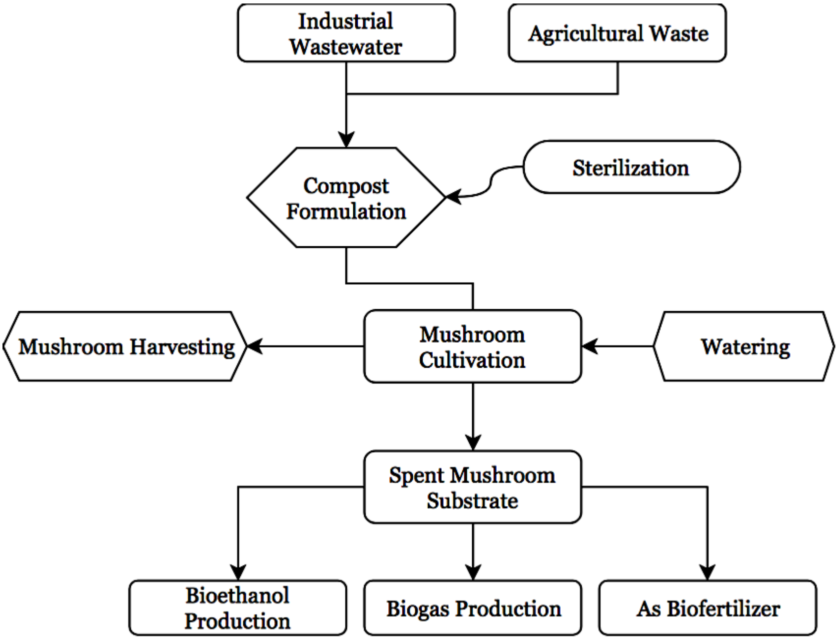

Figure 2. Integrating agro-based industrial wastewater with mushroom cultivation.

Table 2. Industrial wastewaters (effluents) used for the cultivation of mushrooms.

Effluent/wastewater	Substrate	Mushroom species	Reference
Spent beer effluent	Wheat straw	*Pleurotus ostreatus*	Wang *et al*. (2001)
Olive mill wastewater	Wheat straw	*Pleurotus sp*.	Kalmis and Sargin (2004)
Distillery effluent	Wheat straw and bagasse	*Pleurotus florida* Eger (EM 1303), *Pleurotus pulmonarius* (Fries) Quelet (EM 1302) and *Pleurotus sajor-caju* (Fries) Singer (EM 1304)	Pant *et al*. (2006)
Maize wastewater	Wheat straw	*Pleurotus ostreatus* and *Pleurotus floridae*	Loss *et al*. (2009)
Agro-food industry wastes	Wheat straw, cotton waste, and peanut shelf	*Pleurotus sp*., *Lentinula edodes*	Philippoussis and Diamantopoulou (2010)
Olive mill waste	Wheat straw	Seven *Pleurotus* strains	Ruiz-Rodriguez *et al*. (2010)
Distillery wastewater	Sugar cane bagasse	*Pleurotus flabellatus* and *Pleurotus sajor-caju*	Gothwal *et al*. (2012)
Dairy wastewater	Sugar cane bagasse	*Pleurotus flabellatus* and *Pleurotus sajor-caju*	Gothwal *et al*. (2012)
Fruit packaging industry effluent	Wheat straw	*Pleurotus ostreatus*	Karas *et al*. (2016)

Pant *et al.* (2006). Besides this, maize processing wastewater, agro-food industry wastes, olive mill waste, distillery wastewater, dairy wastewater and fruit packaging industry effluent have been successfully tested for the cultivation of certain edible mushroom species (Loss *et al.*, 2009; Philippoussis and Diamantopoulou, 2010; Ruiz-Rodriguez *et al.*, 2010; Gothwal *et al.*, 2012; Karas *et al.*, 2016).

Assets in sustainable development

Besides the benefits of utilizing TAWs in mushroom cultivation, there are a few problems associated with integrating it. There might be the presence of certain heavy metals, toxins, pesticides, persisting aromatic hydrocarbons, etc. Therefore, prior testing and confirmation of their presence are recommended (EPA, 1993). However, the acceptable limits. Table 3 provides Australian Standards recommended by EPA for finished substrate/compost products (Australia Standards, 2012). However, not all kinds of TAWs may contain all these toxic substances in higher amounts, a better example is dairy, bakery and palm oil wastewater, they comprise most of the non-toxic constituents. The EPA recommends that the following Australian Standards be adopted in setting environmental goals and quality parameters for compost products:

- AS 4454–2012 for compost, soil conditioners, and mulches
- AS4419–2003 for foils for landscaping and garden use
- AS 3743–2003 for potting mixes
- AS/NZS 5024 (INT)–2005 for potting mixes, composts, and other matrices: examination for legionellae.

Table 3. Australian Standards recommended by EPA for finished substrate/compost products (Sources: Australian Standards, 2012; EPA, 2019).

Chemical contaminant	Maximum permissible limit
Aldrin	0.02 mg/Kg
dieldrin	0.02 mg/Kg
Arsenic	20.00 mg/Kg
Cadmium	1.00 mg/Kg
Chromium	100.00 mg/Kg
Copper	150.00 mg/Kg
Lead	150.00 mg/Kg
Mercury	1.00 mg/Kg
Nickel	60.00 mg/Kg
Zinc	300.00 mg/Kg
Glass, metal and rigid plastics	0.50 % dry matter (w/w)
Plastics–light and flexible or film	0.05 % dry matter (w/w)

Further utilization of spent mushroom substrates

SMS, which has less lignin due to the digestion process by extra-cellular lignocellulosic enzymes during mushroom production, is merit for biofuel production. The lower lignin content but high nitrogen and ash content make the SMS more easily digested by microbial degraders to yield more reducing sugars. Indeed, the resulting polysaccharides act as a suitable substrate for hydrolysis, since the production of SMS itself has served as a form of pre-treatment. Table 4 provides previously published reports on the utilization of SMS for various purposes. These include biogas production, animal feed for mature castrated male sheep, post-weaning calves feeding, biodiesel production, bioethanol production, reducing sugar production, biofertilizer production, methane production, butanol production, etc. (Kumar *et al.*, 2020).

Conclusion

Mushroom production represents a source of extra income for farmers and can be grown on a diverse range of lignocellulosic wastes including agricultural residues and agro-based industrial wastes. The spent mushroom substrates have great potential for bioenergy production. The left-over material after cultivation can be used for the generation of biogas, biodiesel, and bioethanol, etc. Non-residual and non-fractional materials may also be used as a fed-stock for composting and using as an effective biofertilizer.

Table 4. Spent mushroom substrate (SMS) used for various purposes.

Spent substrate	**Purpose**	**Reference**
Wheat straw	Biogas	Bisaria *et al.* (1990)
Wheat straw	Mature castrated male sheep feeding	Fazaeli and Masoodi (2006)
Cotton waste-based substrate	Biodiesel production	Kwak *et al.* (2009)
Hydrolysates of corncob-based substrate	Bioethanol production	Oguri *et al.* (2011)
Sawdust	Post-weaning calves feeding	Kim *et al.* (2011)
Wheat straw	Sugar production	Kapu *et al.* (2012)
What straw	Biogas production	Sonia *et al.* (2013)
Alkali treated wheat straw	Reducing sugar and biofertilizer production	Zhu *et al.* (2013)
Dairy manure and wheat straw	Modeling of methane production	Shi *et al.* (2014)
Yard trimmings and wheat straw	Biogas production	Lin *et al.* (2014)
Wheat straw	Butanol and biodiesel production	Zhu *et al.* (2016)

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Conflict of interest

The corresponding author on behalf of all co-authors declares that there is no conflict of interest.

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