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Treatment of Pharmaceutical Effluent by *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* Isolated from Spoilt Water Melon

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ABSTRACT

A study was designed to assess the efficacy of yeast isolated from spoilt water melon in the biological treatment of pharmaceutical effluent. Two yeast species were identified as *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*. Each of the yeast was inoculated into the effluent and incubated for 15 days. *Saccharomyces cerevisiae* shows the highest percentage reduction of 52.5, 52.5 and 58.7% for BOD, COD and nitrate respectively of the pharmaceutical effluent and closely followed by the consortium which has 44.5, 44.5 and 72.0% for BOD, COD and nitrate reduction, respectively. The least percentage reduction was displayed by *Torulaspora delbrueckii* with 38.3, 38.3 and 79.7%. The study revealed that *Saccharomyces cerevisiae* isolated from spoilt water melon could be used in the biological treatment of pharmaceutical effluent.

Key words: Water melon, effluent, BOD, COD, nitrate, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, yeast, pharmaceutical, consortium

INTRODUCTION

Effluent is generated by residential, institutional, commercial and industrial establishments. It includes household waste liquid from toilets, baths, showers, kitchens sink and so forth that is disposed of via sewers (Nemade *et al.*, 2009). Pharmaceutical effluents are waste generated by pharmaceutical industry during the process of drugs manufacturing. Pharmaceuticals and personal care products industries suffer from inadequate effluent treatment due to the presence of recalcitrant substances. Some of the most representative pharmaceutical and personal care products found in receiving water including antibiotics, lipid regulators, anti-inflammatories, antiepileptic, tranquilizers and cosmetics ingredients containing oil and grease. The pharmaceutical industry manufactures biological products, medicinal chemicals, botanical products and the pharmaceutical products as well as other commodities. The industry is characterized by a diversity of products processes, plant sizes as well as waste water quantity and quality. In fact, the pharmaceutical industry represents a range of industries with operations and processes as diverse as its products (Christensen, 1998). Hence, it is almost impossible to describe a “typical” pharmaceutical effluent because of such diversity.

The following methods can be applied in treatment of effluent, in general, therefore, selecting a treatment method form industrial effluents depends primarily on the following factors:

- Identifying the various pollutants present in the effluent
- Characterizing the effluent
- Regulating the sewers and separating the waste streams
- Selecting the treatment technology based on the different available physical, chemical or biological treatment capabilities possibility of separating waste streams
- Possibility to carry out local or partial treatment, or recycling
- Probability of secondary pollution incident, even of slight or occasional, that can worsen treatment plant operation (appearance of glues, fibers, oils, sand etc.) (EPA., 2013)

The pharmaceutical industry employs a wide array of waste water treatment and disposal methods. Wastes generated from these industries vary not only in composition but also in magnitude (volume) by plant, season and even time depending on the raw material and the process used in manufacturing of various pharmaceuticals. Hence, it is very difficult to specify a particular treatment system from such a diversified pharmaceutical industry. Many alternative treatment processes are available to deal with the wide array of waste produced from this industry an associated sludge process, trickling filtration, the powdered activated carbon-fed activated sludge process and the anaerobic hybrid reactor. An incomplete listing of the other treatments includes incineration, anaerobic filters, spray irrigation, oxidation ponds, sludge stabilization and deep well injection. Based upon extensive experiment with waste treatment across the industry a listing of the available treatments and disposals is summarized as follows (Drillia *et al.*, 2005):

- Separate filtration or mycelium, drying and recovery of fermentation broth and mycelium for use as animal feed supplements
- Solvent recovery at centralized facilities or at individual sectors, reuse and or incineration of collected solvents
- Special recovery and subsequent sale of sodium sulphate
- Cooling towers for reuse of cooling and jacketing water

In most developing countries like Nigeria, most industries dispose their effluents without treatment. These industrial effluents have a hazardous effect on water quality, habitat quality and complex effects on flowing water (Bound and Voulvoulis, 2005).

In Nigeria, main contributors to the surface and ground water pollution are the by-products of various industries such as textile, metal, dyeing chemicals, fertilizers, pesticides, cement, petrochemical, energy and power, leather sugar processing, mining and others. The discharge of industrial effluents, municipal sewage, farm and urban wastes carried by drains and canals to rivers worsen and broadens water pollution (EPA., 2013).

Generally, pharmaceutical industries do not generate uniform waste streams, due to the variety of medicines produced during any given processing period (Houk and Demarini, 1988). In recent times, a wide variety of pharmaceuticals have been found in fresh and marine waters and some of these compounds are potentially capable of causing harm to both aquatic and terrestrial life form. The presence of pharmaceutical chemicals in the environment is a matter of concern due to their lipophilic and non-biodegradability nature, as well as their biological activities. Coastal water provide homes for an amazing array of plants and animals and are recreational havens for millions of visitors each year. But recently, scientists have raised concerns about pharmaceutical residues detected in rivers and coastal waters and their potential to cause adverse effects in humans and aquatic species (NOAA., 2014). The aim of this study therefore, is to treat pharmaceutical effluent using yeast species isolated from spoiled water melon.

MATERIALS AND METHODS

Description of study area and sample collection: Spoilt water melons were collected from grocery stall in Bosso market, Minna, Nigeria. Pharmaceutical effluent was collected from Peace Standard Pharmaceutical Company, Ilorin, Nigeria. The raw effluent was collected aseptically using a clean 5 L plastic can from the point of discharge into the environment; the company produces anti-malaria, antibiotics, multivitamins, analgesics and anesthetics. The collected materials were kept at 4°C until analysis. The spoilt water melon was collected aseptically using a clean polythene bag and transferred to the microbiology laboratory.

Isolation of yeast: Saboroud Dextrose Agar (SDA) was used for isolation of yeast using pour plate method. One gram of the fleshy part of the spoilt water melon was inoculated into a test tube containing 9 mL of sterile water and serially diluted. One milliliter of the serially diluted sample was plated as described by APHA (2006). The plates were incubated at 25°C for 24-48 h as described by Fawole and Osho (2002). After the incubation period, two different yeast of distinct colony were sub-cultured separately on SDA and incubated at 25°C for 24 h. A stock culture was made for each of the yeast sp. using slant bottle containing SDA and labeled yeast A and yeast B, respectively.

Identification of the yeasts: Characterization of the yeast isolates was based on Gram staining and sugar fermentation. The test organisms were inoculated into fermentation medium containing glucose, sucrose, fructose, mannose, sorbitol, lactose, maltose using phenol red as indicator. Durham's tube was introduced into each of the test tube and incubated at 25°C for 24-48 h. A change in color of the medium from red to yellow indicated acid production while, a displacement at the top of the inverted Durham tube indicated gas production.

Effluent treatment set up: Some portion of the yeast designated as yeast A and yeast B in each of the stock culture were inoculated into Potato Dextrose Broth (PDB) inside a conical flask, respectively and incubated for 48-72 h at 25°C. Hundred milliliters of pharmaceutical effluent was measured into 10 conical flask (250 mL) and labeled as (effluent+yeast A, effluent+yeast B, effluent+yeast A and B) and a control flask without yeast cells. The experiment was set up in triplicate. Five millimeters each of the yeast cells A and B were inoculated into the effluent excluding control. The set up were incubated at 25°C for 15 days.

The following tests were carried out on the effluent at 5 days intervals.

Physicochemical properties of effluent: Saratale *et al.* (2009) and APHA (1998) methods for textile dye effluent treatment were adopted for all the physicochemical properties of the effluent analyzed and the methods were as follows:

Nitrate: Twenty five milliliters of the effluent was dispensed into a reduction column followed by addition of 75 mL NH₄Cl-EDTA solution and mixed. The mixed sample was collected at the rate of 7-10 mL min⁻¹ into original sample flask. After reduction, 2.0 mL color reagent was added to 50 mL sample and mixed. The resulting solution was allowed between 10 min and 2 h. The absorbance was then measured at 543 nm against a distilled water reagent blank.

Phosphate: Fifty milliliters of the effluent was dispensed into 125 mL Erlenmeyer's flask and one drop of phenolphthalein indicator was added. A drop of 5N H₂SO₄ solution was added to discharge

the color. Eight milliliter combined reagent was added, mixed thoroughly was allowed for 10 min. The absorbance was then measured at 880 nm, using reagent blank as the reference solution.

Sulphate: Hundred milliliters of the effluent was dispensed into 250 mL Erlenmeyer's flask. 0.2-0.3 g of BaCl₂ crystals was added and stirred for 1 min. After stirring, the sample was placed in 5 cm cuvette for 4 min and the absorbance was measured at 420 nm.

Turbidity: Turbidity was measured in Nephelometric Turbidity Units (NTU). The turbidity meter was ON and clean, prepared calibration standards cal 800 NTU was inserted and read. The first one was removed and the second one cal 200 NTU was inserted and read. After the second one was read and removed, the third one cal 100 NTU was inserted and read. The sample cell was filled with the sample and inserted into sample holder. Enter key was pressed and the turbidity value was read and recorded.

Chemical oxygen demand: In dichromate reactor digestion method was used, small volumes of the effluent sample (2 cm³) was pipetted into vials containing the premeasured reagents, including catalysts and chloride compensator. The vial was incubated at 150°C for 2 h for digestion to take place and allowed to cool. The COD measurement is made by HACH 890/DR Colorimeter.

Biochemical Oxygen Demand (BOD₅)

- **Determination of initial DO:** Initial DO was determined using DO meter
- **Incubation:** Incubation of bottle containing desired sample (90 mL) was done at 20±1°C
- **Determination of final DO:** Determined with DO meter

Calculation:

$$\text{BOD}_5 \text{ (mg L}^{-1}\text{)} = \frac{D_1 - D_2}{P}$$

Where:

D₁ = DO of diluted sample immediately after preparation (mg L⁻¹)

D₂ = DO of diluted sample after 5 days incubation at 20°C (mg L⁻¹)

P = Decimal volumetric fraction of sample used (mL of the sample taken (90 mL) divided by total volume of the BOD bottle (250 mL))

RESULTS AND DISCUSSION

Physicochemical properties of pharmaceutical effluent using *Saccharomyces cerevisiae*:

The results in Table 1 show the physicochemical properties of pharmaceutical effluent treated with *Saccharomyces cerevisiae* within a period of 15 days. The phosphate, sulphate, turbidity, total dissolve solid and conductivity of the pharmaceutical effluent fall within the acceptable limits allowed for waste water discharge by World Health Organization (WHO) and Environmental Protection Agency (EPA) standard. The value ranged from 1.63, 5.82, 8.0, 18.0 mg L⁻¹, 8.93-13.10 NTU, 320-345 mg L⁻¹ and 477-520 µs cm⁻¹ for sulphate, turbidity, TDS and

Table 1: Physicochemical properties of pharmaceutical effluent treated with *Saccharomyces cerevisiae*, *Torulasporea delbrueckii* and consortium

Parameters	Days			
	Initial	5	10	15
<i>Saccharomyces cerevisiae</i>				
Nitrate (mg L ⁻¹)	134	43.9	37.2	55.4
Phosphate (mg L ⁻¹)	3.7	2	1.6	5.82
Sulphate (mg L ⁻¹)	100	8	22	18
Turbidity (NTU)	320	13.10	11.74	8.93
Conductivity (μs cm ⁻¹)	380	511	520	477
TDS (mg L ⁻¹)	254	345	349	320
BOD (mg L ⁻¹)	3840	2648	2956	1824
COD (mg L ⁻¹)	9600	6620	7390	4560
<i>Torulasporea delbrueckii</i>				
Nitrate (mg L ⁻¹)	134	46.1	24.7	27.2
Phosphate (mg L ⁻¹)	3.7	1.01	1.45	2.28
Sulphate (mg L ⁻¹)	100	5	16	12.00
Turbidity (NTU)	320	14.97	7.5	13.54
Conductivity (μs cm ⁻¹)	380	524	569	476
TDS (mg L ⁻¹)	254	352	382	319
BOD (mg L ⁻¹)	3840	2568	2568	2368
COD (mg L ⁻¹)	9600	6420	6420	592
Consortium				
Nitrate (mg L ⁻¹)	592	50.1	43.7	37.5
Phosphate (mg L ⁻¹)	3.7	1.57	1.93	1.82
Sulphate (mg L ⁻¹)	100	14	40	14.0
Turbidity (NTU)	320	25.3	12.44	16.0
Conductivity (μs cm ⁻¹)	380	571	630	489
TDS (mg L ⁻¹)	254	381	444	333
BOD (mg L ⁻¹)	3840	2836	2240	2152
COD (mg L ⁻¹)	9600	7090	5600	5330

BOD: Biochemical oxygen demand, COD: Chemical oxygen demand, TDS: Total dissolve solid

conductivity, respectively. While, the BOD, COD and nitrate values are exception and their values was quite high beyond recommended limits. They range from 1824-2956, 4560-7390 and 37.2-55.4, respectively. The study revealed the physicochemical quality of the pharmaceutical effluent. The initial physicochemical properties were observed to be very high, the value are; 134, 3.7, 100 mg L⁻¹, 320NTU, 380 μs cm⁻¹, 254, 3840 and 9600 mg L⁻¹ for nitrate, phosphate, sulphate, turbidity, total dissolve solid, conductivity, BOD and COD, respectively. The reduction rate of the physicochemical properties by the yeast was rapid at the initial time of the treatment with a sharp reduction in the properties at day 5 this could be due to the removal of organic load from the effluent and definitely the toxicity as stated by Ong *et al.* (2012). However in day 10, there was increase in some of the physicochemical properties like sulphate, total dissolve solid, conductivity and biological oxygen demand in *Saccharomyces cerevisiae* treated effluent. This may be due to saturation of the organism binding site with such properties and death of some of the organism but there was reduction in some of the physicochemical properties like nitrate, phosphate, Chemical Oxygen Demand (COD) and turbidity. This could be due to the yeast activities present in the medium. The BOD, COD, nitrate value were higher than WHO (2011) recommended standard; a similar result of the toxic effect of pharmaceutical effluent was observed by Adekunle and Eniola (2008).

Physicochemical properties of pharmaceutical effluent using *Torulasporea delbrueckii*:

The results in Table 1 show the physicochemical properties of pharmaceutical effluent treated with *Torulasporea delbrueckii* within the period of 15 days. The nitrate, phosphate, sulphate, turbidity, total dissolve solid and conductivity of the pharmaceutical effluent fall within the acceptable limits

allowed for waste water discharge by World Health Organization (WHO) and Environmental Protection Agency (EPA) standard and they range from 24.7-46.1-1.01- 46.10-5.0 and 16.0 mg L⁻¹, 7.5-14.97 NTU, 319-382 mg L⁻¹, 476-569 μs cm⁻¹, respectively. While, BOD and COD values are exception and their values was quite high beyond recommended limits. They range from 2368-2568, 5,920 and 6420 mg L⁻¹, respectively. The physicochemical parameters were carried out using APHA (1998) technique.

Physicochemical properties of pharmaceutical effluent with yeast consortium: The results in Table 1 show the physicochemical properties of pharmaceutical effluent treated with the consortium of *Saccharomyces cerevisiae* and *Torulasporea delbrueckii* within the period of 15 days. The nitrate, phosphate, sulphate, turbidity, total dissolve solid and conductivity of the pharmaceutical effluent fall within the acceptable limits allowed for waste water discharge by World Health Organization (WHO) and Environmental Protection Agency standard (EPA) and they range from 37.5-50.1, 1.57-1.93, 14-40 mg L⁻¹, 12.44-25.3 NTU, 333-444 mg L⁻¹, 381-630 μs cm⁻¹, respectively. While, the BOD and COD values are exceptional and their values was quite high beyond recommended limits. They range from 2132-2836, 5330-7090 mg L⁻¹, respectively. The physicochemical parameters were determined using APHA technique. The presence of high value of BOD and COD will result into the impairment of the oxygen content available in the water body for living organisms. The reaction between *Saccharomyces cerevisiae* and *Torulasporea delbrueckii* as compared to the result obtained showed that an antagonistic type of reaction might have taken place between them.

The result in Fig. 1 shows the physicochemical properties of pharmaceutical effluent treated with *Saccharomyces cerevisiae*, *Torulasporea delbrueckii* and the consortium respectively at day 15 and were compared in order to ascertain the best. *Saccharomyces cerevisiae*, was found to be the best with 52.5, 52.5 and 58.7% reduction of BOD, COD and nitrate, respectively, closely followed by their consortium which has 44.48, 44.48 and 72.0% as the percentage reduction for BOD, COD and nitrate respectively and the least percentage reduction was displayed by *Torulasporea delbrueckii* which has 38.3, 38.3 and 79.70% for BOD, COD and nitrate, respectively.

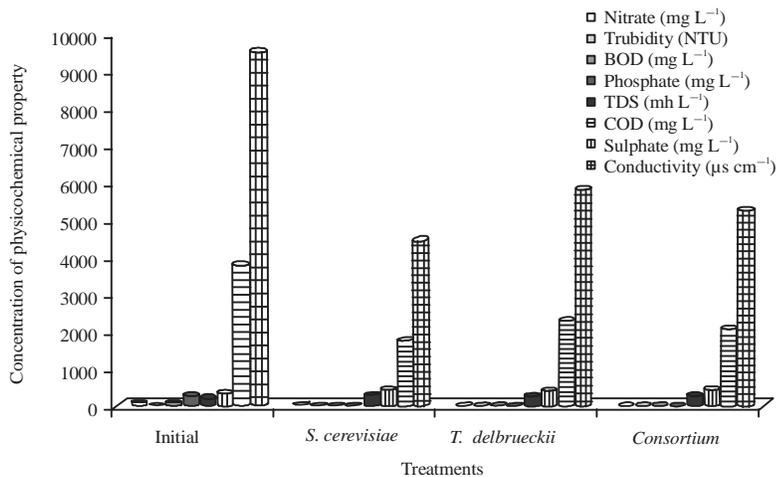


Fig. 1: Comparison of the physicochemical properties of pharmaceutical effluent by *Saccharomyces cerevisiae*, *Torulasporea delbrueckii* and their consortium

The reaction between *Saccharomyces cerevisiae* and *Torulaspota delbrueckii* as compared to the result obtained showed that an antagonistic type of reaction might have taken place between them.

The two yeast isolates (*Saccharomyces cerevisiae* and *Torulaspota delbrueckii*) possess biodegradation ability which was revealed by the reduction of the initial high value of BOD, COD, nitrate, sulphate and turbidity.

There was a significant reduction in physicochemical properties (Fig. 1), this could be as a result of the mechanism of biodegradation which depends in part, on the compounds being degraded, as it is well known that microorganisms can degrade and even completely mineralize many reactive dyes under certain conditions and the products of intermediate metabolism produced during the decolorization process, can be degraded by the enzymes produced by the bacteria (Wang *et al.*, 2008). The yeasts showed considerable colour removal which could be as a result of the adsorption of the anionic dyes in the effluent which may be due to the nitrogen containing (or protein) group that comprises the yeast biomass and other cellular components as suggested by Kumar *et al.* (2007). All the isolates worked best at neutral pH of 7.0. *Saccharomyces cerevisiae* reduce the BOD value from 3840-1824 mg L⁻¹, COD from 9600-4560 mg L⁻¹, nitrate, from 134-55.4 mg L⁻¹, sulphate from 100-18.0 mg L⁻¹, turbidity from 320-8.93 NTU. *Torulaspota delbrueckii* degrades the BOD level from 3840-2368 mg L⁻¹, COD from 9600-5920 mg L⁻¹, nitrate, from 134-27.2 mg L⁻¹, sulphate from 100-12.0 mg L⁻¹ and turbidity from 320-13.54 NTU.

The consortium was able to degrade the BOD level 3840-2132, COD from 9600-5330, nitrate from 134-37.5, sulphate from 100-14.0 and Turbidity from 320-16.01. The high value of BOD indicate that a high amount of oxygen is needed by the yeast isolates while, stabilizing decomposable organic matter under aerobic condition in order to carry out redox reaction. The turbidity level reduces due to the biodegradation action of the yeast isolates and this makes the pharmaceutical effluent to become clearer. The offensive odour of the effluent disappears after the treatment process.

CONCLUSION

The pharmaceutical effluent contain high amount of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and nitrate which pose a great hazard to both human and aquatic environment when released untreated in to the environment. Yeast (*Saccharomyces cerevisiae* and *Torulaspota delbrueckii*) are capable of biological treatment of pharmaceutical effluent, although *Saccharomyces cerevisiae* show a higher degradation efficacy, making it a better alternative over *Torulaspota delbrueckii*.

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