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Use of fungi in bioremediation of palm oil mill effluent (POME).*

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ABSTRACT

This work is aimed at assessing the bioremediation potentials of *Candida rugosa and Geotricum candidium* in Palm oil mill effluents. The physicochemical qualities of the POME sample used in this work showed average COD (105000 mg/l), BOD (16300mg/l), oil and grease (7515 mg/l), TDS (8020.25 mg/l) and TSS (18740.25mg/l).
Geotricum candidium and *Candida rugosa* were among eleven fungal strains isolated from palm oil mill effluent, two of which only had the highest ability to degrade oil and grease. They were identified and used for bioremediation of the effluent by optimizing some of the cultural conditions during remediation process. Amongst the four nitrogen sources (Soybean, groundnut meal, urea and yeast extract) used soybean best supported bioremediation of palm oil mill effluent by both *Candia rugosa* and *Geotrichum candidium* with BOD of 71.8 and 79.7%, COD 80.7% and 49.1% respectively. Similarly oil and grease reduction was 85.2% in *candida rugosa* and 83.6% in *Geotrichum* treated POME. Studies on the effect of carbon source on the bioremediation of POME showed that *Candida rugosa* tremendously used glucose that other carbon source with 81.5%(BOD), 92.1%(COD) , 84.9% (O&G) and 83.8%(TSS) reductions. This may be attributed to its importance in the initiation of fungal growth. Corn starch also best supported bioremediation using *Geotrichum candidium* with 74.5% (BOD), 85 %(COD), 70%(O&G) and 83.5%(TSS) removal. Cassava starch showed the least support in both fungal degraded POME. The importance of agitation in cell growth will never be emphasized hence the use of different agitation speed in this study. Maximum organic load reduction was obtained at 300rpm with BOD (77.6%) ,COD (89.9%), O&G(86.9%) and TSS(90.5%) reduction in *Candida rugosa* treated POME while 250rpm agitation speed showed BOD(87.4%), COD(91.9%), O&G(86.2%) and TSS(94.3%) reduction in *Geotrichum candidium* treated POME . This work shows bioremediation potentials of *Candida rugosa and Geotricum candidium* in the treatment of POME , therefore suggest that these fungi can be used to reduce pollution effect caused by indiscriminate disposal of this effluent in the environment.

Keywords : BOD, Bioremediation, COD, degradation, Fungi ,Oil and grease, Pollution

1 INTRODUCTION

World oil palm cultivation has been majorly dominated by Indonesia and Malaysia while Colombia, Thailand and Nigeria are cultivating to lesser extent (Ohimain et al 2013a). In Nigeria, about 80% traditional palm oil processing is mostly carried out manually in home and cottage industries, using undeveloped equipment and semi-mechanized processors thereby making the process labour intensive. Most mechanized processing mills are located mainly in southern Nigeria where oil palm trees are found both in the wild and plantations. Palm oil mill effluents (POME) are discharges of oily and viscous highly polluting wastewater from palm oil mill industries. POME has posed major sources of land and aquatic pollutants when discharged directly into the environment where these industries are sited. Because of their nature POME forms layer on the surface of body of water where it is discharged and causes decrease in oxygen transfer rate in the aerobic system. It has been reported that the discharge of untreated POME into the ecosystem leads to the loss of biodiversity, soil deterioration and pollution of waterways (Awotoye *et al*, 2011). It is reported that the resultant effect of POME is due to presence of phenols and other organic acids that produce both phytoxic effect and antibacterial activity (Pascual *et al* 2007]. However, these polyphenolic fractions degrade over time and forms in manure to soil (Piotrowska *et al,* 2006).Therefore, bioconversion of POME to organic manure can be a veritable tool for its disposal only when its effects on the ecological properties are known.

POME has been noted to contain high organic load, extensive amounts of plant nutrients and forms cheap source of plant nutrients when biodegraded (Kittikun *et al* 2000, Onyia *et al* 2001, Pechsuth, M. *et al.,* 2001]. POME pollution is associated with high concentration of organic matter (COD= 40000- 50000 mg/L, BOD= 20000-25000 mg/L) (Najafpour *et al*., 2006), oil and grease and contains complex polymers (carbohydrates, protein, lipids, minerals and nitrogenous compounds (Ohimain *et al*, 2013a). POME is a naturally thick, brownish liquid containing colloidal slurry of water (95 – 96%), oil (0.6 – 0.7%), total solid (4 – 5%), suspended solid (2 – 5%), temperature (80-90 °C), acidic (pH 3.8-4.5) (Onyia *et al*., 2001, Ahmad et al., 2005). The nature of POME causes odour pollution (Er *et al.,* 2011). Biological treatment is a high efficiency method to eliminate organic matter, suspended solids and oil. Many group of hydrocarbon degraders and lipase producing microorganisms such as lipolytic bacteria, fungi and mold flourish on POME (Izah and Ohimain, 2013b). Various reports have shown that POME contains micro and macro

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nutrients as part of its mineral composition (Ohimain *et al.* 2012d, Wood et al. 1979, Borja *et al.* 1996). Microorganisms mineralize these nutrients through the activities of their enzymes. POME microorganisms have found application in bioconversion of POME into useful and demanding products. POME is reported to be useful in antibiotics, solvent, biofertilizer, biohydrogen, bio-insecticides, organic acids, polyhydroxyalkanoates and enzymes productions (Wu *et al.* 2009).

The pollution caused by palm mill industries in Nigeria especially in the southern part of the country has posed a great source of worry to the Federal Ministry of Environment as the organic load randomly discharge to nearby community streams are higher than World Health Organization maximum permissible limits (Chikogu et al2012). This therefore has prompted the urgent need to find a way to preserve the environment.

Biological treatment of POME has been widely studied and found to be the most efficient method. Some aerobic organisms like a tropical marine yeast (*Yarrowia lipolytica)* NCIM3589 have been used to study degradation of POME using in a lagoon(Oswal *et al.,* 2002), trickling filter(TF) (Nik Norulaini *et al.*, 2001) and rotating biological contactors(RBC)(Najafpour *et al.,* 2005). Aerobic degradation of POME is used to stabilize effluent quality and reduce high level of organic load in it. This work is aimed at assessing the bioremediation potential of some fungi isolates (*Candida rugosa and Geotricum candidium*) during POME treatment.

2 MATERIALS & METHOD

2.1 Sample collection

Fresh palm oil mill effluent samples were collected from a local palm oil mill factory - Starline Palm Oil mill industries Umukalika, Obingwa, Abia State Nigeria. Samples were collected using sterile screw cap 500 ml glass bottles, transferred immediately to the laboratory and stored at 4 ^{o} C before further analysis.

2.2 Physicochemical properties of Palm Oil Mill Effluent (POME)

Some physicochemical characteristics of the POME were analyzed according to standard methods (APHA, 1998) in order to monitor the biodegradation process. The Biological Oxygen Demand (BOD_5) was determined by manometric method with respirator(OXI TOP IS6) and Chemical oxygen Demand (COD) determined using(COD meter HACH). Turbidity of the POME samples were determined using Unicam8625 spectrophotometer at 340nm wave length. Total Kjeldahl nitrogen was as described by Ademoroti (1996) using Kjeldahl indophenols colorimetric method (Unicam8625 spectrophotometer) at wavelength of 635nm and values extrapolated from standard calibration curve. Total suspended solids (TSS) and total dissolved solids (TDS) were determined using portable hand held meter (HANNA-DIST 1 HI 991002). Temperature and pH of the effluent sample were measured using HANNA pH/ORP/Temperature meter (HI 991002, HANNA Instrument, Romani). Oil and grease determination was carried out using gravimetric method after soxhlet extraction (APHA, 1998)

2.3 Microbial analysis

2.3.1 Characterization and identification of fungi

The populations of fungi isolates in the samples were enumerated using serial dilution pour plate method of Pepper and Gerba (2004), Benson (2002). About 0.1ml of POME sample was serially diluted in sterile physiological saline and aliquots of the dilutions were ascetically plated into MEA containing 50 mg/ml chloramphenicol in order to prevent bacterial contamination. The agar plates were incubated inverted at 28°C for 3-5 days for fungi culture plates. After incubation, the fungal colonies that grew on the medium were characterized based on general principles of fungal classification (mycelium colours and growth patterns), macroscopic and microscopic morphology (Samson et al. 1984 , Molla *et al.,* 2002). For the microscopic morphology, a drop of ethanol was placed on a clean slide with the aid of the sterile forceps, fragment of pure culture was transferred into the ethanol on the slide and a drop of lactophenol blue stain was added .The ethanol was allowed to evaporate (Enemuor et al., 2012;) then slides covered with a cover slip and viewed under the microscope.

2.4 Effect of carbon source on bioremediation of POME using fungal isolates

The method used was as described by (Wu et al2006, Ibegbulam-Njoku et al 2013) with little modification in POME. Experiment was determined to elucidate the effect of carbon source additive on the effluent degradation. Raw POME(100ml) was used in combination with 2%(w/v) of the respective carbon source: glucose, potato starch, cassava starch, corn starch and corn meal inoculated with 0.1ml (10^6cells/ml) and incubated at 28^{o}C for 144h on a rotary shaker at 180rpm.Samples were drawn and tested for BOD_5, COD ,TSS and O&G.

2.5 Effect of nitrogen sources in bioremediation of POME using fungal isolates

The effects of nitrogen source in bioremediation of POME was determined as previously described by (Wu et al 2006 and Ibegbulam-Njoku et al 2013). Different nitrogen sources (soybean, Groundnut meal, Urea, yeast extract, NH_4Cl and NH_4SO_4) were used, each at concentration of 1% (w/v) into 250ml conical flask containing 100ml of POME, and incubated at 30^{o}C for seven days. Samples were drawn and tested for BOD_5, COD, TSS and O&G.

2.6 Effect of agitation in Bioremediation of POME using fungal isolates.

The method used was described by Husseiny (2008). Two sets of Erlenmeyer flasks (250ml) containing media were inoculated and one set placed on a rotary shaker, while the other set was static. The effect of agitation rate on bioremediation resulting from growth of isolated organisms in palm oil mill effluent was studied at 50,100,150,250,350 and 400 rev/min respectively. All were incubated at 28^{o}C for 144h. The agitation rate was varied by increasing the rotation speed of the shaker. Samples were drawn and tested for growth at BOD_5, COD, TSS and O&G.

3 RESULT & DISCUSSIONS:

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Physicochemical properties of palm oil mill effluent

The physicochemical characteristics of all samples of POME collected were determined and revealed acidic pH range of 3.74 to 4.57, temperature of 32-38°C , O&G 6700-8400mgl, TDS of 7614 - 8420mg/l and TSS was 17650 - 19450 mg/l. COD showed high values between 70000-130000mg/l, BOD values 12000 - 18200mg/l, nitrogen content (TKN) of 780- 1400mg/l and turbidity of 20559-22687 NTU (Table1).The results of pH, BOD,COD and O&G ranges are all in line with reports Pechsuth et al 2001, Ahmed *et al.*, 2004, Choorit *et al*., (2007) and Phutdhawong *et al.*, 2008 but contrast the reports of Oswal *et al*.(2002) , Ahmad *et al*.(2006) , Vijayaraghavan *et al*. (2007) and Bhatia *et al*., 2007; The high BOD **is** associated with constitution of palm tree fruits and processing technique. The high organic matter is due to the presence of different sugars like as arabinose, xylose, glucose, galactose and manose at the concentrations of 6.43, 0.44, 0.22, 0.15 and 0.10% dry weight, respectively (Agamutha and Tan, 1986). The TDS result differs from the 20,500mg/l findings of Ahmad *et al* (2006) but compares favourably with TSS reports of previous studies in palm oil mill effluent ranging between16500-19500 mg/l (Ahmad *et al*., 2003, Najafpour *et al*., 2006; mohammad *et al*., 2007).the total Kjeldahl Nitrogen(TKN) is in line with findings of Pechsuth *et al*., 2001.

Table1. Physicochemical properties of Palm Oil Mill Effluent (POME)

Properties	Sample A	Sample B	Sample C	Sample D	Mean ± Std.
COD(mg/l)	130000	70000	130000	90000	105000±260
BOD(mg/l)	17000	18200	18000	12000	16300±252
TSS(mg/l)	19450	17650	19208	18653	18740.25±692
TDS (mg/l)	8117	7614	7930	8420	8020.25±292
O&G (mg/l)	7300	7660	8400	6700	7515±615
Turbidity(NTU)	22687	22574	20559	21945	21941.25±846
TKN (mg/l)	1400	780	900	1080	1040±233
pH	3.74	4.54	3.93	4.57	4.20±0.37
Temperature °C	38	34	36	32	35±2.24

Cultural identification of fungi from palm oil mill effluent.

In this study, eleven fungal isolates were isolated from the palm oil mill wastewaters (table 2). The eleven fungal isolates were identified as *Candida cylidracae, Penicillium digitatum, Penicillium italicum,, Aspergillus niger, Aspergillus terreus, Geotricum candidium, Mucor racemosus, Rhizopus stolonifer, Saccharamyces cerevisea, Fusarium* sp,*Candida rugosa*. The morphological characteristics of the isolates are shown in Table 2. The presence of *Penicillium digitatum, Penicillium italicum, and Rhizopus stolonifer* in POME is in line with previous reports of Kathubutheen (1981) and Molla *et al* (2002). *Aspergillus niger, Aspergillus oryzae* and *Mucor racemosus* were also reported to be associated with palm oil mill effluent (Wood,1977, Molla *et al*., 2002, Ohimain *et al*., 2012e). *Aspergillus niger* is a natural flora of soil and associated with degradation of organic matter. Studies on *Candida* sp. have shown that it is readily isolated from palm tree and palm fruits and might explain the presence of *Candida rugosa* in palm oil mill effluent. *Candida* sp is said to be involved in degradation of lignin and cellulose also it was grown in olive oil wastewaters (D'Annibale *et al*., 2006). Similarly *Geotrichum candidum* has been reported as a lipase producing organism (Ghosh *et al*., 1996). Among all the fungal species isolated *Geotrichium candidium and Candida rugosa* were further selected for biodegradation of the POME.

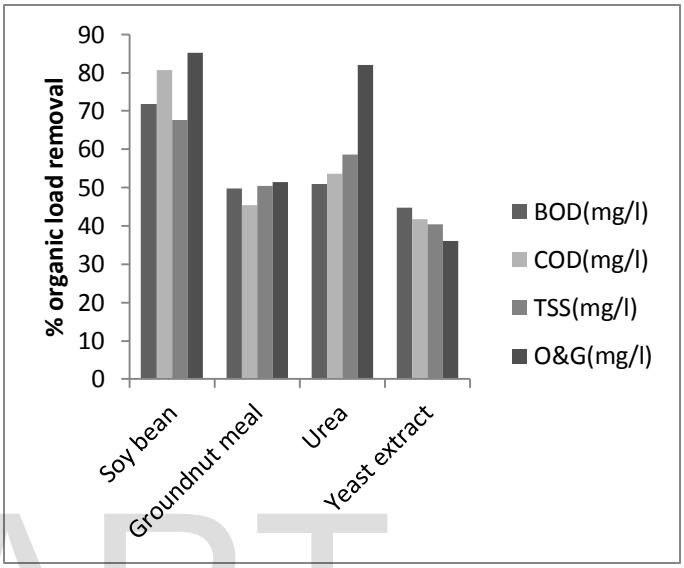

Figure 1 Effect of nitrogen sources on bioremediation of POME by *Candida rugosa* isolates.

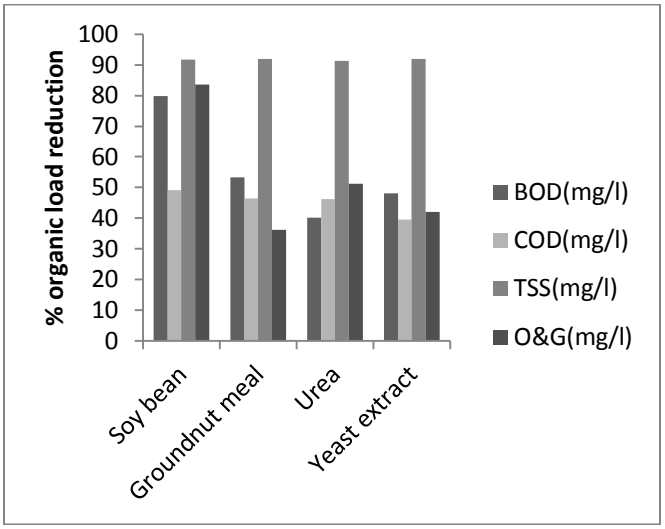

Figure 2 Effect of nitrogen source on bioremediation of POME by Geotrichum candidium isolates.

Table 2: Cultural identification of fungi from palm oil mill effluent

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Sample	Cultural Characteristics	Microscopy	Identification
A,B,D,C2	White-velvety to powdery with central tuft 3cm in 7days darken after about 2wks	Singly on aerial hyphae or terminally in groups of 2-3 short conidiophores cylindrically often with a slightly swollen base	*Candida cylidracae*
B,C,D,C2	Yellowish-Greenish colonies ,diameter 6 cm within 7 days with conidiophore .	Ellipsoidal – cylindrical conidia dark green in mass. Phialids were often solitay	*Penicillium digitatum*
A,,B,C	Diameter of 7cm in 7days white smooth colony with sweet odour	Advancing hyphae was septated and cylindrical conidia formed when fertile hyphae broke	*Geotricum candidium*
A,B,C,D	Rapidly growing creamy to brownish mycelia with dark brown to conidial head consisting mostly dense felt of erect conidiophores and often appears golden on the reverse side.	Long conidiophores stipes with smooth wall and septate	*Aspergillus niger*
A,B,D,	Colonies with diameter of 1.5cm within 7days. White conidia heads later changed to creamy but in fresh isolates were often shiny	Conidiophores stipe hyaline to slightly yellowish smooth-walled	*Aspergillus terreus*
B,C,D	White – creamy glubose colonies .	Globose and ovoid shaped cells in chains forming psuedomycelium	*Saccharamyces cerevisea*
B,D,C2	Whitish colony with irregular shaped fluffy sporangiophores becoming brownish-grey with age	Showed tall sporangiophore with sporangium Sporangiophores showed smooth walled and barrel shaped when young.	*Mucor racenosus*
A,B,C.D	Colony became blackish-brownish with age. Sporangiophores were 1.5-4mm tall solitary and some in group of 2-7	smooth or slightly rough-walled stolons opposite the branched rhizoids was observed. Sporangiophores was irregular in shape often polygonal or ovoid	*Rhizopus stolonifer*
A,B,C.D	Colonies showed whitish-cream colonies on PDA with diameter of 5.4cm in 4days at 25°C dark pigmented conidiophores were branched with single phialides and fatty spores.	Whitish –yellowish brown on reverse view.Septate banana shaped conidia	*Fusarium* sp
A,C,D	White wrinkled color colony that showed resistant to nystatin	Short pseuohyphae	*Candida ru*
A,B,C,D	Colonies showed diameter of 3cm within 12-14days,colour was grey-greenish with aromatic odour.	Conidia showed ellipsoid-cylindrical metulae bearing 3-6 slender phialides.	*Penicillium italicum*

Table 2 : Cultural identification of fungi from palm oil mill effluent (Contld)

Effect of nitrogen sources in bioremediation of POME using fungal isolates

The effect of nitrogen source in palm oil mill effluent remediation was studied using various organic and inorganic sources of nitrogen (Fig 1&2). It was observed the nitrogen source had a remarkable effect on organic load reduction of POME. In general, organic reduction by the POME supplemented with soybean was significantly ($P < 0.05$) higher than those supplemented with other nitrogen sources with best activity of 71.8% BOD, 80.7% COD, 85.2% O&G and 67.6% TSS reduction *in Candida rugosa* treated POME(Fig1).Similarly, soy bean supplemented POME best supported the organic load removal using *Geotricum candidium* in the treatment showing 79.8% BOD,49.1% COD, 83.6%O&G and 91.8%TSS removal. However, *Candida rugosa* and *Geotrichum candidium* did not utilized yeast extract and Urea effectively in their respective treatment. Addition of small quantity of soybean and groundnut meal was said to improve degradation of palm oil sludge. Similarly, Salleh *et al.* (1993) reported lipase production by *Rhizopus oryzae* in medium containing soy digest.

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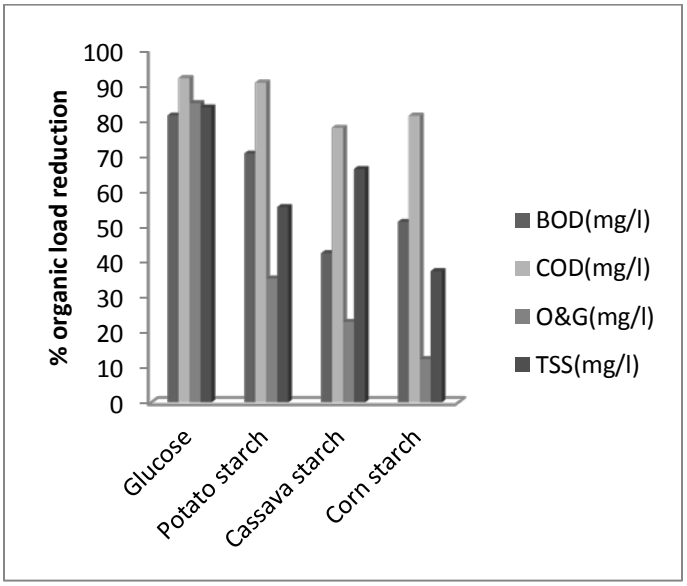

Figure 3. Effect of carbon source on bioremediation of POME by Candida rugosa isolates

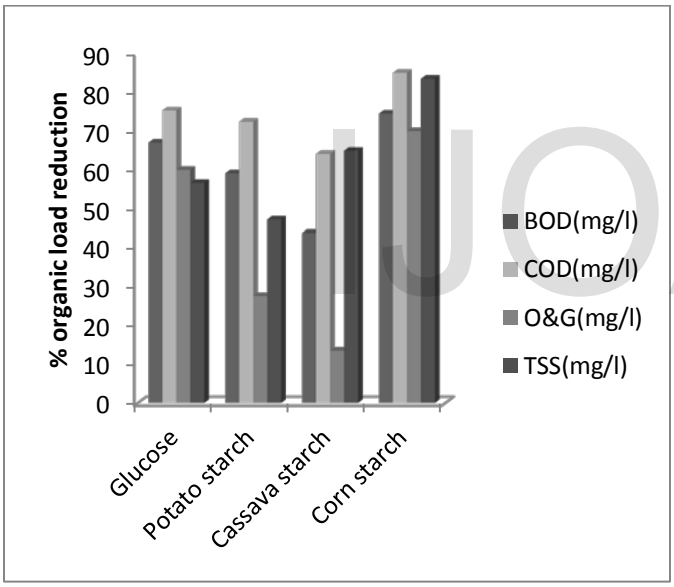

Figure 4. Effect of carbon source on bioremediation of POME by *Geotrichum candidium* isolates.

Effect of carbon sources on biodegradation of POME using fungal isolates.

Four carbon sources, consisting of glucose, cassava starch, potato starch and corn starch evaluated for their effect on the organic load reduction in fig 3& 4. The highest BOD reduction of 81.5% was obtained by *candida rugosa* in glucose and the least in cassava starch 42.4%.Similarly, reductions of 92.1% COD , 84.9% O&G ,83.8%TSS values were also obtained with glucose supplementation (Fig 3). Previous studies had reported glucose(carbon source) to initiate fungal growth and support biodegradation of many industrial effluent such as pulp and paper mill effluent (Hossain et al.,2001, Pant *et al.*, 2008).However, *Geotrichum candidium* showed best degradation in corn starch supplemented POME treatment with BOD(74.5%),COD(85%), O&G (70%) and TSS(83.5%) removal while cassava starch showed the least support for degradation of POME (Fig 4). Organic carbon source (Corn meal) was reported to yield the best support for lipase production by *Candida rugosa* (Song *et al.*, 2001) also Chen et al 2013 reported the use of cornstarch to best improve biodegradation of alkaline wastewater.

Table3.Effect of agitation on bioremediation of POME using fungal isolates.

Isolates	Agitation speed rev/min	BOD(mg/l)	COD(mg/l)	O&G(mg/l)	TSS(mg/l)
C. rugosa	0	14609^{g}	85150^{g}	6865^{e}	11035^{h}
	50	14790^{g}	86400^{h}	6875^{e}	14785^{i}
	100	14000^{f}	86200^{h}	6707^{e}	7607^{f}
	150	13390^{e}	75070^{f}	4860^{d}	10634^{h}
	200	12090^{e}	56240^{e}	3540^{c}	8105^{g}
	250	8600d	32600^{d}	2209^{b}	4125^{e}
	300	3650^{a}	10600^{a}	986^{a}	1785^{a}
	350	4300^{b}	15890^{b}	3370^{c}	2791^{b}
	400	4820^{c}	18400^{c}	3445^{c}	3798^{c}
G. candidium	0	14782^{i}	98500^{i}	7040^{e}	17108^{h}
	50	12480^{h}	82800^{h}	6076^{d}	16909^{g}
	100	11862^{g}	76500^{g}	5900^{c}	10885^{f}
	150	9600^{f}	53900^{f}	5760^{c}	8886^{e}
	200	8320^{e}	47000^{e}	5700^{c}	3296^{d}
	250	2059^{a}	8500^{a}	1040$_{a}$	1065^{a}
	300	4870^{b}	9100^{b}	1390^{a}	1755$_{b}$
	350	5300^{c}	11800^{d}	3370^{b}	2491^{c}
	400	6425^{d}	10956^{c}	3488^{b}	2966^{d}

a, b ,c, d, e means with different superscripts are significantly different (P<0.05).

Effect of agitation

Agitation is imperative for proper oxygen transfer and mixing of nutrients in fermentation system (Fadzilah et al 2010). It is also required for cells to fully utilize nutrients including oxygen in aerobic culture (Najafpour 2007) so as to create most favourable environment in a system. Table 3 shows the effect of agitation speed (50–400 rev/ min) on bioremediation of palm oil mill effluent. Agitation was provided by orbital shaker. The agitation speed affected both the organic load reduction and oil removal. The maximum organic load reduction was observed at 300rpm with highest BOD (77.6%) ,COD (89.9%), O&G(86.9%) and TSS(90.5%) reduction obtained by *Candida rugosa* while the least performance was at 50rpm with BOD(9.3%), COD(17.7%), O&G(8.5%) and TSS(21%)reduction. However, at 250rpm degradation was at its peak with 87.4% (BOD), 91.9%COD, 86.2% (O&G) and 94.3% (TSS) reduction of organic load reduction by *Geotrichum candidium* treated POME with least activity at 50rpm. Previous studies on the effect of agitation speed on lipase production from oil wastewater revealed that 125 and 200 rpm favoured lipase production in respective cases (Zouaou *et al.*, 2012 , Balan *et al* ., 2012) .Similarly, the effect of agitation in palm oil mill effluent using 300 rpm of stirring speed was found to be most conducive for cell growth (Fadzilah *et al* 2010).However previous

reports had noted that higher agitation rate can reduce cell growth due to shear stress and heterogenous mixing effects (Nadeem *et al* 2009).

CONCLUSION:

The optimal benefits remain the primary factor for manufacturing operation hence in POME treatments various methods need to be assessed for their economic appeal. Bioremediation has continued to be the best process of treating most industrial effluent. In this study, *Geotrichum candidium* and *Candida rugosa* have shown the capability of improving palm oil mill effluent under controlled cultural conditions. Therefore, seminars, symposia and public lecture can be organised by the Government for small / medium scale industries thereby creating awareness on how to improve POME quality before discharge in the environment.

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