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Biodegradation of Cellulosic Materials by Marine Fungi Isolated from South Corniche of Jeddah, Saudi Arabia

Fuad Ameen*, Mohamed A. Moslem, Sarfaraz Hadi and Ahmed E. Al-Sabri

Botany and Microbiology Department, College of Science,
King Saud University. Riyadh, Saudi Arabia.

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Twenty eight fungal isolates belonging to 12 genera were derived from debris, sediment and water samples collected from *Avicennia marina* stands 25km south of Jeddah city on the Red Sea coast of Saudi Arabia. Eight of these isolates were found to be able to grow in association cellulosic waste materials under in vitro conditions in the absence of any carbon source. Isolates were further tested for their potential to degrade paper and clothes wastes by co-cultivation under aeration on a rotary shaker. These fungi accumulated significantly higher biomass, produced ligninolytic and cellulase enzymes, and liberated larger volumes of CO_2. These observations indicated that the selected isolates were able to breakdown and consume the waste materials.

Keywords: Biodegradation, enzyme activity, waste materials, mangrove.

Saudi Arabia's rapid industrialization, construction, and urbanization have increased levels of pollution and waste. Since the beginning of the seventies of the twentieth Century, the Kingdom is seeing an unprecedented economic expansion that lead to a massive expansion of the urban areas of the Kingdom[1].In the Kingdom of Saudi Arabia context, the municipal solid waste collection services are facing an increasing number of problems such increasing population growth, changes in habits and lack of awareness of the impact of solid waste on the environment[2]. Jeddah is the second largest city in Saudi Arabia located on the eastern coast of the Red Sea and is the major urban center of western Saudi Arabia[3]. The city has a population of more than 3.5 million. With the population increase, the amount of sewage became a major problem as the capacity of the sewage treatment plants is largely insufficient and much of the raw sewage (~146,000 m^3/day, [4] is dumped into the coastal area creating a dramatic environmental impact[5-10].

The cellulosic materials present in intermediate- and some low-level wastes (cotton, paper and wood) are composed of a range of polymeric material, which reflects their origins and the degree of processing[11].

Wood contains significant amounts of both cellulose and hemicelluloses whereas the hemicellulose content of paper is much lower (<10%) and depends on the raw material used (hard or soft wood)[12, 11].

Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule[13, 14]. Various types of microorganisms, including bacteria, fungi and algae, play a key role in the biodegradation of wastes, due to their highly reproductive ability, effective metabolism of the organic pollutants as well as their biological diversity[15]. Most of the studies have been primarily

* To whom all correspondence should be addressed.
E-mail: alfouad2004@hotmail.com

focused on bacteria, but a few studies have shown that pollutants could be degraded in soil by fungi[16- 18]. Fungi are well known to degrade, or cause deterioration to, a wide variety of materials and compounds, processes known as mycodegradation and mycodeterioration, respectively[19].

Fungi, including the ligninolytic and non-ligninolytic fungi, are usually considered to be adaptive and endurable against higher concentration pollutants in the environment than bacteria. They play a significant role of degradation of persistent pollutants[20].

In the present study, several fungal genotypes were isolated from the debris and sediments of *Avicennia marina* stands on the Red Sea coast near Jeddah city, Saudi Arabia. Potential of these isolates to degrade paper and clothes waste materials and enzymes involved in this activity were investigated.

MATERIALS AND METHODS

Collection of samples

Debris floating in the *Avicennia marina* stands 25km south of Jeddah city on the Red Sea coast was collected along with the Sea water in sterilized specimen tubes. Additionally, soil sediment from the same stands was also collected. The debris consisted of dead fallen leaves, pieces of pneumatophores, bark and wood. The samples were brought to lab for isolation of fungi.

Isolation of fungi

Fungi from solid debris were isolated by splitting the specimen into smaller pieces and directly laying on a synthetic medium formulated to simulate seawater conditions based on the compositions of [21-23] with some modification. Composition of the medium per liter was: $MgSO_4$ 246.5mg, $FeSO_4.7H_2O$ 5.56mg, $ZnSO_4.7H_2O$ 0.29mg, $MnSO_4.H_2O$ 0.34mg, $CuSO_4.5H_2O$ 0.025mg, NH_4Cl 5.35g, KCl 7.46g, $CaCl_2.2H_2O$ 1.47g, NaCl 5.84g, $COCl_2.6H_2O$ 0.027mg, KH_2PO_4 136mg, Na_2MoO_4 24mg with pH adjusted to 5.5. The medium was gelled with 15 g agar per liter. For the sake of convenience, the medium was tentatively designated as 'MF1'. Isolation of fungi from water accompanying the specimen was done by serial dilution. Ten-fold dilutions were laid on 'MF1' medium. For isolation from sediments, also dilution technique was used. One gram of sediment was suspended in 10 ml of sterilized distilled water; and tenfold dilutions thereof were spread on the same medium. The plates were incubated at 30 ± 2º C for 5-7 days.

Identification of the fungi

Pure fungal isolates were established from the initial plates using standard procedures of fungal cultivation. Fungal strains were examined under the microscope and were identified according to the procedures of [24-27].

Fungal growth and biomass accumulation on cellulosic waste

In all, 28 isolates derived from different types of samples were cocultivated with Paper and clothes pieces to test activity on theses fibers. 'MF1' broth devoid of dextrose or any other source of carbon was used for the tests. Cellulytic waste pieces of 1 cm2 were cut from sterilized paper and clothes. Single pieces were precisely weighed and then transferred to 150 ml conical flasks containing 100 ml sterilized 'MS1' broth. Fungal discs of 6 mm diameter were punched out from the culture plates with the help of sterilized steel tube. Each flask was inoculated with a single disc of an individual isolate. The flasks were incubated at 30 ± 2º C for 4 weeks in a rotary shaker gyrating at 100 rpm. Three replicate flasks were maintained for each fungal isolate. For each treatment, three flasks with corresponding inoculum but without paper and clothes, pieces were retained as controls. Biomass accumulation of those isolates which showed growth in association with paper and clothes waste was estimated in comparison with the controls after four-week incubation. Cellulosic waste pieces with or without fungal growth were dried in the oven at 90ºC overnight and were weighed precisely again. Gain in biomass under each treatment and the corresponding control was recorded by subtracting the initial weights; and difference between gain in treatment and control was considered to be due biodegradation activity of the fungus.

Enzyme activity assays

Eight fungal isolate which showed biomass accumulation under treatment were co-cultivated with paper and clothes pieces in replicates along with the controls as above. As a separate treatment, consortium of the eight above isolates was also tested for enzyme activity. After four weeks of incubation, enzyme level in the

medium was assayed for controls and treatments. Cultures were centrifuged at 10,000 rpm, 4°C; the pellet consisting of fungal cells was discarded and enzyme level was determined in the extracellular fluids. Laccase was estimated by the oxidation of 2, 2-Azino-bis-3-ethyl-benzthiazoline-6-sulfonic acid (ABTS) according to[28, 21], using 0.1 mM ABTS in the reaction buffer of 100 mM sodium tartrate (pH 4.5) with 50 µl culture filtrate. One unit (U) of laccase activity was defined as the production of 1 ¼mol product per min at 30°C and pH 4.5. Manganese-dependent peroxidase (MnP) was estimated by using 0.01% phenol in the presence of 0.1 mM H_2O_2 and 1 mM $MnSO_4$ in 100 mM sodium tartrate (pH 4.5); while lignin peroxidase (LiP) was determined by the oxidation of 2 mM veratryl alcohol in 100 mM sodium tartrate (pH 4.5) with 0.4 mM H_2O_2 [29, 30].

Cellulase activity was determined at 40°C by incubating 1 ml of enzyme solution with 1 ml 0.1 M potassium phosphate pH 8.0 containing 1% (w/v) cellulose powder. The amount of reducing sugars was determined according to[31] procedure. All enzymes assayed in this study were expressed as U/ml.

Estimation of CO_2 evolution

Fungal isolates, paper and clothes pieces were co-cultivated as above and quantity of CO_2 evolved was estimated for an incubation period of 4 weeks. Three flasks were maintained for each isolate together with corresponding control flasks inoculated with the fungus but devoid of substrate.Volumetric and gravimetric estimation of CO_2 evolved during 4- week incubation was performed using strum test. For gravimetric analysis sterile air was sequentially passed through1M KOH solution to remove atmospheric CO_2 and then through the flasks under assessment. The bubbling air provided aeration for the fungal activity and at the same time allowed any CO_2 evolving from the fungal activity to dissolve readily in the broth. The test was performed at room temperature (26 ± 2°C). Amount of CO_2 dissolved in the broth was estimated by adding 100 ml of 0.1 M $BaCl_2$ which formed precipitate of barium carbonate; and CO_2 released was gravimetrically calculated by measuring the weight of the precipitate. Difference in the values obtained between control and test bottles were recorded. For volumetric analysis, the dissolved carbon dioxide present in the medium was estimated by titration. The broth was filtered to remove fungal mass and the waste material substrate and 25 ml filtrate was taken in a conical flask to which 0.05ml of 0.1N thiosulphate solution was added. After the addition of 2 drops of methyl orange indicator, solution was titrated against 0.02M sodium hydroxide solution. End point appeared as change in color from orange red to yellow. After these two drops of indicator was added and titration was continued until, a pink color was observed. Volumes of the titrant used were noted and the amount of CO_2 evolved was calculated using the formula: [A x B x 50 x 1000] ÷ V; Where A = volume of NaOH titrant in ml, B = normality of NaOH, and V = volume of sample in ml.

RESULTS

Growth of Fungal Isolates on pieces of Paper

The biodegradation ability of the selected fungal strains was individually analyzed by their growth on pieces of paper in MSM. Fungal growth was visible to the naked eye on the surface of media after four weeks treatment period (Fig. 1).

Biomass accumulation by fungi under co-cultivation with Paper.

During the fungal treatment of paper as a cellulosic waste, inocula of eight isolates gradually developed association with the substrate and within 10-15 days grew to form colonies of variable size and appearance. In the absence of paper substrate (controls) none of the isolates, including *Alternaria alternata* (Figure 1A), showed any appreciable growth of the inocula. On the other hand, as shown in Figures 1B-D, profuse growth of *Alternaria alternata, Aspergillus terreus* and *Eupenicillium hirayamae* respectively was noticed around the paper pieces.

These isolates showed greater accumulation of biomass as compared to their corresponding controls (Table 1). *Eupenicillium hirayamae* gained the maximum weight of (56.3%) followed by *Alternaria alternata* (43.9%)); whereas minimum weight gain (20.4%) was recorded in *Geosmithia pallida*.

Enzyme activity of fungal isolates with paper substrate

Production of laccase, MnP, LiP and Cellulase enzymes was recorded in most of the

Table 1. Biomass accumulation by fungal isolates during co-cultivation with paper substrate as sole carbon source

Fungal Isolate	Biomass accumulation in 4 weeks		Gain in biomass due to
	Control* (g l^{-1})	Treatments* (g l^{-1})	biodegradation[g l^{-1} - (%)]
Alternaria alternata	0.642 ± 0.010	0.924±0.091	0.282 (43.9%)
Aspergillus terreus	0.625 ± 0.011	0.862±0.050	0.237 (37.9%)
Cladosporium sphaerospermum	0.599± 0.048	0.822±0.084	0.223 (37.2%)
Geosmithia pallida	0.382± 0.012	0.460±0.053	0.078 (20.4%)
Paecilomyces formosus	0.524 ± 0.005	0.601±0.083	0.135 (25.7%)
Eupenicillium hirayamae	0.591±0.049	0.924±0.050	0.333 (56.3%)
Byssochlamys spectabilis	0.594 ± 0.038	0.778±0.138	0.184 (30.9%)
Eupenicillium rubidurum	0.555 ± 0.010	0.697±0.059	0.142 (25.5%)
Consortium	0.662±0.027	1.054±0.131	0.392 (59.2%)

*Data represents mean of three replicates ± Standard Deviation.

control and treatment cultures of the eight tested fungi as well as their consortium (Table 2). Consortium of the eight tested fungi showed remarkably higher level of enzymes as compared to the individual fungi under both, control and treatment conditions. This may be due to synergistic effect of the pooled genotypes. *Eupenicillium hirayamae* produced high level of Cellulase (29.71± 6.9 U ml^{-1}) followed by *Alternaria alternata* with Cellulase (20.66±3.81 U ml^{-1}) which corroborates well with biomass accumulation in the same order (Table 1).

CO_2 evolution due to fungal activity on Paper pieces

No significant difference was observed between CO_2 evolution estimates taken by volumetric and gravimetric methods; therefore, data was merged as means of the two procedures for each fungal isolate (Table 3). *Eupenicillium hirayamae* showed maximum enhancement of Co_2 emission over the control (87.2%) followed by *Alternaria alternata* (64.7%) and *Aspergillus terreus* (49.6%).

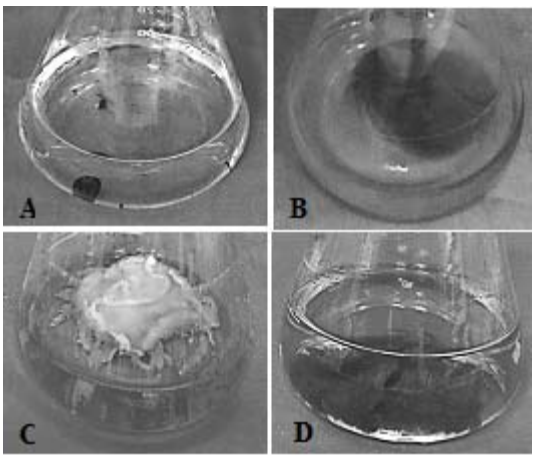

Fig. 1. Co-cultivation of fungi with paper substrate. (A) Control (without carbon source): *Alternaria alternata* – Showing meager growth of fungus in the absence of paper pieces. (B-D) Treatments (with paper fibers): *Alternaria alternata, Aspergillus terreus and Eupenicillium hirayamae* respectively – showing profuse growth of fungus in association with pieces of paper

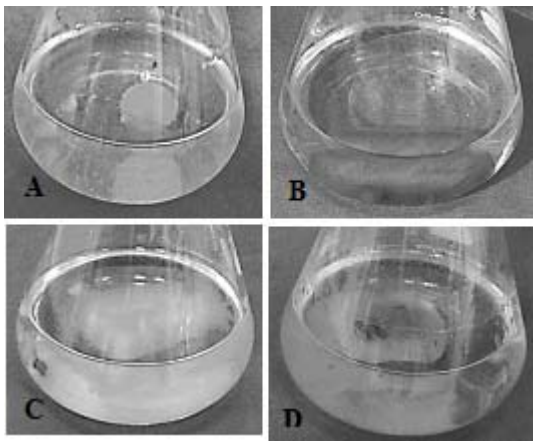

Fig. 2. Co-cultivation of fungi with clothes as carbon source. (A) Control: *Eupenicillium hirayamae* – Showing little growth of fungus without clothes substrate. (B-D) Treatments: *Alternaria alternata, Aspergillus terreus and Eupenicillium hirayamae* respectively – showing abundant growth of fungus in association with pieces of clothes

Table 2. Enzyme activity of fungal isolates during co-cultivation with LDPE film

Fungal Isolate	Controls*				Treatments*			
	LAc (U ml^{-1})	Mnp (U ml^{-1})	Lip (U ml^{-1})	Cellulase (U ml^{-1})	LAc (U ml^{-1})	Mnp (U ml^{-1})	Lip (U ml^{-1})	Cellulase (U ml^{-1})
Alternaria alternata	2.6±0.84	6.15±1.58	ND ND ND	3.34±1.75	3.40±0.56	8.46± 0.83	ND ND ND	20.66±3.81
Aspergillus terreus	1.6±0.61	1.51±0.73	1.56±0.65	1.75±0.53	2.47±1.02	2.15±0.17	1.71±0.54	16.06±1.92
Cladosporium sphaerospermum	2.34±1.20	ND ND ND	1.87±1.08	2.42±0.54	3.1±1.08	ND ND ND	3.53±0.91	14.85±1.01
Geosmithia pallida	ND ND ND	1.95±0.28	0.94±0.17	1.62±0.76	ND ND ND	2.39±1.0 9	1.73±0.47	3.83±0.67
Paecilomyces formosus	2.4±0.65	3.92±0.63	ND ND ND	0.82±0.27	2.2±0.71	3.52±0.6 4	ND ND ND	5.66±0.84
Eupenicillium hirayamae	ND ND ND	2.56±0.53	2.38±0.75	2.53±0.94	ND ND ND	4.29± 0.35	3.15±0.38	29.71±6.9
Byssochlamys spectabilis	ND ND ND	1.78±0.63	ND ND ND	ND ND ND	ND ND ND	1.94± 0.86	ND ND ND	9.66±1.56
Eupenicillium rubidurum	2.4± 0.66	ND ND ND	0.74±0.25	ND ND ND	2.41±0.81	ND ND ND	1.84±0.25	7.23±0.80
Consortium	9.8±2.48	20.27±2.9	6.04±1.95	10.03±2.9	10.71±1.3	22.5±1.75	7.34±0.63	78.34±9.22

* Data represents mean of three replicates ± Standard Deviation.
ND: Not detected

Dry weight of fungi under co-cultivation with clothes

During the fungal treatment of clothes pieces as a sole carbon source, inocula of eight isolates gradually developed association with the substrate and within 15-20 days grew to form colonies of variable size and appearance. In the absence of clothes substrate (controls) none of the isolates, including *Eupenicillium hirayamae* (Figure 2 A), showed any appreciable growth of the inocula. On the other hand, as shown in Figures 2 B-D, improvident growth of *Byssochlamys spectabilis, Alternaria alternata, and Eupenicillium hirayamae,* respectively was noticed around the clothes pieces. These isolates showed greater cumulating of biomass as compared to their compatible controls (Table 4). *Eupenicillium hirayamae* gained the maximum weight of (52.6%) followed by *Alternaria alternata* (45%)); whereas minimum weight gain (18%) was recorded in *Geosmithia pallida*.

Enzyme assay of fungal isolates with clothes substrate

Production of laccase, MnP, LiP and Cellulase enzymes was recorded in most of the control and treatment cultures of the eight tested fungi as well as their consortium (Table 5). Consortium of the eight studied fungi showed exceptionally higher level of enzymes as compared to the individual fungi under both, control and treatment conditions. This may be due to interacting effect of the contributed genotypes. *Eupenicillium hirayamae* produced high level of Cellulase (23.77± 5.88 U ml^{-1}) followed by *Alternaria alternata* with Cellulase (18.89±4.05 U ml^{-1}) which corroborates well with dry weight accumulation in the same order (Table 4).

Table 3. CO_2 evolved during co-cultivation of fungal isolates With Paper pieces

Fungal Isolate	CO_2 evolution in 4 weeks		CO_2 enhancementdue to
	Control* (g l^{-1})	Treatments* (g l^{-1})	biodegradation[g l^{-1} - (%)]
Alternaria alternata	0.460 ± 0.003	0.758±0.063	0.298 (64.7%)
Aspergillus terreus	0.435 ± 0.005	0.651±0.122	0.216 (49.6%)
Cladosporium sphaerospermum	0.432±0.018	0.633±0.024	0.201 (46.5%)
Geosmithia pallida	0.399±0.020	0.448±0.024	0.049 (12.2%)
Paecilomyces formosus	0.466 ± 0.010	0.549±0.044	0.083 (17.8%)
Eupenicillium hirayamae	0.448±0.045	0.839±0.070	0.391 (87.2%)
Byssochlamys spectabilis	0.445 ± 0.004	0.555±0.080	0.11 (24.7%)
Eupenicillium rubidurum	0.452 ± 0.006	0.533±0.028	0.081 (17.9%)
Consortium	0.493±0.043	0.957±0.132	0.464 (94%)

* Data represents means of the estimates via volumetric and gravimetric methods.

Table 4. Biomass accumulation by fungal isolates during Co-cultivation with Clothes pieces

Fungal Isolate	Biomass accumulation in 4 weeks		Gain in biomassdue to
	Control* (g l^{-1})	Treatments* (g l^{-1})	biodegradation[g l^{-1} - (%)]
Alternaria alternata	Control*(g l^{-1})	Treatments*(g l^{-1})	0.29 (45%)
Aspergillus terreus	0.625 ± 0.011	0.883±0.027	0.258 (41.2%)
Cladosporium sphaerospermum	0.599	0.832±0.069	0.233 (38.8%
Geosmithia pallida	0.382	0.453±0.036	0.07 (18%)
Paecilomyces formosus	0.524 ± 0.005	0.626±0.025	0.102 (19%)
Eupenicillium hirayamae	0.591	0.902±0.054	0.311 (52.6%)
Byssochlamys spectabilis	0.594 ± 0.038	0.732±0.052	0.138 (23.2%)
Eupenicillium rubidurum	0.555 ± 0.010	0.668±0.024	0.113 (20.3%)
Consortium	0.662	1.078±0.076	0.416 (62.8%)

*Data represents mean of three replicates ±Standard Deviation

Table 5. Enzyme activity of fungal isolates during co-cultivation with pieces of Clothes

Fungal Isolate	Controls*				Treatments*			
	LAc (U ml^{-1})	Mnp (U ml^{-1})	Lip (U ml^{-1})	Cellulase (U ml^{-1})	LAc (U ml^{-1})	Mnp (U ml^{-1})	Lip (U ml^{-1})	Cellulase (U ml^{-1})
AAlternaria alternata	2.6±0.84	6.15±1.58	ND ND ND	3.34±1.75 6	4.5± 0.53	8.89± 2.02	ND ND ND	18.89± 4.05
Aspergillus terreus	1.6±0.61	1.51±0.73	1.56±0.65	1.75±0.53	2.6±0.70	2.56±0.80	2.69±0.77	12.23±1.11
Cladosporium sphaerospermum	2.95 0.96 3.13	ND ND ND	3.05 0.93 1.64	2.42±0.54	2.59± 1.41	ND ND ND	3.26± 0.73	10.48± 0.82
Geosmithia pallida	ND ND ND	1.63 2.12 ND	1.13 0.84 ND	1.62±0.76	ND ND ND	2.39± 1.09	1.73± 0.47	2.65± 1.18
Paecilomyces formosus	2.4±0.65	3.92±0.63	ND ND ND	0.82±0.27	2.26± 1.18	3.47± 0.81	ND ND ND	2.95± 0.09
Eupenicillium hirayamae	ND ND ND	3.12 2.51 2.07	2.22 3.20 1.73	2.53±0.94	ND ND ND	3.17± 0.24	3.26± 0.74	23.77± 5.88
Byssochlamys spectabilis	ND NDND	1.78±0.63	ND NDND	ND NDND	ND NDND	2.59± 0.778	ND NDND	7.96±1.09
Eupenicillium r ubidurum	2.4± 0.66	ND ND ND	0.74±0.25	ND ND ND	2.62± 0.54	ND ND ND	2.25± 1.09	3.66±0.56
Consortium	9.8±2.48	20.27±2.9	6.04±1.95	10.03±2.96	9.52±0.84	21.58±2.27	7.06±1.2	55.17±5.79

* Data represents mean of three replicates ± Standard Deviation.
ND: Not detected

CO_2 evolution due to fungal activity on clothes

No significant difference was observed between CO_2 evolution estimates taken by volumetric and gravimetric methods; therefore, data was merged as means of the two procedures for each fungal isolate (Table 6). The maximum increment of CO_2 was recorded by *Eupenicillium hirayamae* over the control (69.8%) followed by *Alternaria alternata* (61%) and *Aspergillus terreus* (46.8%).

This trend closely matches with the trends of biomass accumulation and enzyme activity suggesting that variable degree of biodegradation of cellulose substrate has taken place by fungal activity reflected in corroborative levels of enzyme production, biomass accumulation, and CO_2 liberation.

Table 6. CO_2 evolved during biodegradation of pieces of clothes by fungal isolates

Fungal Isolate	CO_2 evolution in 4 weeks		CO_2 enhancementdue to
	Control* (g l^{-1})	Treatments* (g l^{-1})	biodegradation[g l^{-1} - (%)]
Alternaria alternata	0.460 ± 0.003	0.742±0.124	0.282 (61%)
Aspergillus terreus	0.435 ± 0.005	0.639±0.116	0.204 (46.8%)
Cladosporium sphaerospermum	0.432±0.018	0.629±0.045	0.197 (45.6%)
Geosmithia pallida	0.399±0.020	0.436±0.023	0.037 (9.2%)
Paecilomyces formosus	0.466 ± 0.010	0.547±0.043	0.081(17%)
Eupenicillium hirayamae	0.448±0.045	0.761±0.089	0.313 (69.8%)
Byssochlamys spectabilis	0.445 ± 0.004	0.583±0.035	0.138 (31%)
Eupenicillium rubidurum	0.452 ± 0.006	0.541±0.042	0.089 (19.6%)
Consortium	0.493±0.043	0.871±0.070	0.378 (76.6%)

* Data represents means of the estimates via volumetric and gravimetric methods.

DISCUSSION

In the current study, it was found that eight fungi tested could degradation of cellulosic waste at varying degree, also showed that the biodegradation correlated significantly positive with the biomass accumulation of theses isolates. Several other studies, which claimed to demonstrate biodegradation potential of different fungi, have also reported biomass accumulation under similar conditions[32], have reported a significant gain in fresh weight of *Aspergillus niger* and *Rhizopus stolinifer* on 10% kerosene pollution (with 0.530 gm dry weight of mycelia after 7 days growth), and the dry weight of *R.stolinifer* reached to 0.522 gm.[33], DaLuz *et al*. (2013) demonstrated high biomass gain by *Pleurotus ostereatus* cultures on oxo-polymer film indicating biodegradation activity of the fungus.

Different fungi have varying capacities to decompose cellulose because of different enzymatic machinery[34]. This was quite evident from the present study, which explains irregular pattern of cellulose decomposition not only among the different genera but also between different species of same genus (Tables 2 and 5).

Milala *et al*.[35] used rice husk, millet straw, guinea corn stalk and sawdust as fermentation feed substrate for the evaluation of cellulase activity secreted by *Aspergillus candidus*. The ability of cellulolytic fungi isolates, *Trichoderma viride*, *Aspergillus niger* and *Fusarium oxysporum* to degrade cellulosic waste materials *viz*., paddy straw, sugarcane bagasse and banana stalks was collaborate with fungal enzymes[36]. Several studies by[37] have been carried out to produce cellulolytic enzymes from biowaste degradation process by many microorganisms including fungi such as *Trichoderma*, *Penicillium* and *Aspergillus* species. Release of CO_2 during co-cultivation of fungi and waste materials in broth is considered a reliable indicator of biodegradation[38], and its estimation is considered a standard method for assaying the biodegradation activity[39]. Vanishree *et al.*,[40], studied the amount of CO_2 released during biodegradation of petrol substrate as indicator for

activity of Aspergillus sp. Balba *et al*., [41], stated that mineralization studies involving measurements of total CO_2 production could provide excellent information on the biodegradability potential of hydrocarbons in contaminated soils.

This is the first research isolating and documenting high diversity of marine fungi from mangrove species *Avicennia marina* on the south Corniche of Jeddah on Red Sea Coast of Saudi Arabia. The eight fungal isolates derived from mangrove stands may make promising candidates for further investigations regarding their ability to degrade and feed on cellulosic materials from contaminated environments.

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