

Research Article

Biodegradation of Aliphatic-aromatic Copolyester under Thermophilic Conditions

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Abstract: The biodegradation of poly (tetramethylene adipate-co-tetramethylene terephthalate) (BTA-copolyester) as synthetic polyester was investigated under thermophilic conditions. Two efficient BTA degrading actinomycetes were isolated from compost at thermophilic phase. These strains were identified as *Thermobifida fusca* and *Thermobispora bispora*. The degradation rate for BTA films within 7 days was 17.12 and 16.96 mg/week.cm² by *T. fusca* and *T. bispora*, respectively. The optimum BTA40:60 degradation conditions are obtained as pH7 and 55°C. The both strains exhibited a wider substrate spectrum as they are able to degrade synthetic polyesters (BTA40:60, PCL-S MaterBi ZF03U/A) and natural polymers (poly-β-hydroxybutyric acid (PHB) and carboxymethyl cellulose). It was shown that the extracellular hydrolyases activity from the both strains was induced in the presence of BTA-copolyester, while the presence of additional carbon sources such as glucose or a complex medium suppressed enzyme formation. Tributyrin as triglycerides was degraded by the both crude concentrated BTA-hydrolases. In contrast the enzyme was not capable to depolymerize the natural polymers PHB and carboxymethyl cellulose, although the organism itself degraded both types of polymers. The obtained results showed that the degradation rate with *T. fusca* BTA40:60-hydrolase was 3.67 mg/day.cm² and was 3.5 mg/day.cm² with *T. bispora* BTA40:60-hydrolase. The pH optimum for BTA-hydrolases was 7 with 20 and 100 mM phosphate buffer and it was 6 with 150 mM citrate buffer. Finally, it could be concluded that actinomycetes and their hydrolases play an outstanding role in recycling of biodegradable plastics under thermophilic phase during composting process.

Keywords: Actinomycetes, Biodegradation, Bioplastic, enzymatic degradation, thermophilic conditions

INTRODUCTION

Synthetic plastics have been widely used as basic materials in different industries because of their excellent characteristics and low cost. Most synthetic plastics wastes are incinerated or buried. However, incineration of synthetic plastics waste, in particular, brings about secondary environmental pollution due to the generation of poisonous gases (such as dioxin emission from PVC incineration) and corrodes the incinerator inner walls with the enormous heat generation. Because of the low density of the majority of synthetic plastics, the volume fraction of plastics waste in the municipal solid waste is far greater than the weight fraction and plastics waste takes up a lot of landfill space. Plastics, which makes up about 5-8 % of the municipal solid waste, remains undegraded in the environment for such a long time that it causes serious environmental problems (Jendrossek *et al.*, 1993; Witt *et al.*, 1997; Kim *et al.*, 2000). Egypt generated about 16 million tons of Municipal Solid Waste (MSW) in 2006 and plastics amount Post-Consumer Waste (PCW) reaches about 6% /year of the total MSW (Plastics Technology Center, 2008).

Plastic materials have shown to be very resistant to environmental influences such as humidity or microbial

attack. This is especially, the case for polymers like polyethylene, polypropylene or polystyrene, which exhibit a backbone solely built of carbon atoms. However, other polymers with heteroatoms in the main chain are potentially susceptible to hydrolytic cleavage of the, e.g., ester bonds or amide bonds. Initiated by the increasing problems with plastics waste during the last decade, polymers have been developed, which can undergo a controlled biological degradation (Amass *et al.*, 1998). It is very an important to find a solution and prevent accumulation of thousands tons of solid waste every year.

Biodegradable plastics opened the way for new waste management strategies since these materials are designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities. Most of the plastics on the market, claimed to be biodegradable, are based on synthetic and microbial polyesters (Augusta *et al.*, 1992; Witt *et al.*, 1997; Eubeler *et al.*, 2010; Nowak *et al.*, 2011a; Belal, 2013a). Polyesters are potentially biodegradable due to the hydrozable ester bonds. In addition, they combine several properties that make them attractive candidates for various industrial applications. Cellulose and starch as natural polymers are degraded simply by microorganisms under environmental conditions.

Within the natural polymers is polyhydroxybutyrate (PHB), which are produced intracellularly by various microorganisms. The PHB was available on the market under trade name "Biopol". The commercial polyesters product of Biopol was recently stopped, probably due to the high price level of this microbial polyester and the variation in PHB properties is limited. Beside the natural polyester PHB, a number of the synthetic aliphatic polyesters such as poly (ϵ -caprolactone) (PCL) are produced as commercial products. Aliphatic polyesters exhibit usually good biodegradability, but as e.g., PCL they exhibit a significant disadvantage- the low melting temperature of about 60°C-excluding it from many applications (Witt *et al.*, 1997; Müller *et al.*, 1998)

In contrast to most aliphatic polyesters, aromatic polyesters like poly (ethylene terephthalate) (PET) provide excellent material properties and, hence, are commercially widely used (e.g., bottles). However, up to now these polymers are considered as resistant against microbial attack (Huang, 1989; Aminabhavi *et al.*, 1990; Kawai, 1995; Müller *et al.*, 2001; Nowak *et al.*, 2011b). With the intention of combining both, biodegradability and good material properties, copolyester containing aliphatic as well as aromatic monomer was tested as biodegradable materials. The combinations of terephthalic acid (30 to 60%), adipic acid and 1,4-butanediol (BTA-polymers) turned out to be the most appropriate combination both, with regard to the material properties (flexible films, melting point from 90-140°C) (Witt *et al.*, 1995).

In 1998, the BASF AG in Germany started to produce a BTA-based copolyester on a commercial scale, which is now on the market under the trade name Ecoflex®. This product exhibits properties similar to polyethylene and the price is quite low for the raw material (3-4 €/kg). The Eastman company (USA) is announcing another BTA-modification; the product is called 'Eastar Bio'. These two aliphatic-aromatic copolyesters overcome the disadvantages of many aliphatic materials (Müller *et al.*, 2001; Witt *et al.*, 2001). The degradation of this co-polyester has been investigated in different conditions (Kleeberg *et al.*, 1998; Witt *et al.*, 2001; Belal, 2003; Trinh *et al.*, 2008). Biodegradable plastics can be degraded into water and carbon dioxide by microorganisms has attracted attention recently. Biodegradable plastics lend themselves to microbial degradation, however, studies on the degrading microorganisms, in particular biochemical and enzymologic research on such degrading microorganisms, has been relatively sparse (Tokiwa *et al.*, 1976; Pranamuda *et al.*, 1995; Uchida *et al.*, 2000; Belal, 2003).

Composting is the most promising method of treating such biodegradable plastics. A thermophilic microorganisms isolated from compost was found to have a higher BTA and PCL-degradability (Kleeberg *et al.*, 1998; Nakasaki *et al.*, 2006). Since composting at

high temperature is an ideal method for recycling biodegradable plastics by thermophilic microorganisms (Tokiwa *et al.*, 2009), isolation of thermal resistant BTA copolyester and PCL-degraders is desired. Among the various thermophilic microorganisms, some actinomycetes have been shown to produce enzymes that hydrolyze polyesters at high temperature (Klingbeil *et al.*, 1996; Tomasi *et al.*, 1996; Kleeberg *et al.*, 1998; Calabria and Tokiwa, 2004; Tokiwa and Calabria, 2004). Therefore, the present work was designed to isolate, characterize and evaluate thermophilic microorganisms in degradation of aliphatic-aromatic copolyesters from compost in thermophilic phase during composting process and using their in degradation other bioplastics under different conditions.

MATERIALS AND METHODS

Bioplastic materials: The aliphatic-aromatic copolyester (1, 4-butanediol, terephthalic acid and adipic acid) (BTA 40:60) and PCL-S MaterBi ZF03U/A (ϵ -caprolactone and starch (40%)) were provided by the Lab of Prof. Deckwer and Dr. Müller, Gesellschaft für Biotechnologische Forschungen (GBF, Braunschweig, Germany). Both types of polyesters were available as sheets (film). Using punches with defined diameters films of defined surface areas were cutted out. PHB as microbial bioplastic was extracted from *Rhizobium elti* in my previous study (Belal, 2013b) and it was available as powder.

Sterile circular films of the polyester 2.5 cm in diameter were used in the degradation tests on agar plates or in liquid medium. The surface area was 4.91 cm² when the films were treated with the microorganisms in one surface or in case of using the films in the degradation test on agar plates but it was 9.82 cm² when the films were treated with the organism for the both surface film or in case of using the films in the degradation test in liquid medium.

Plastic films sterilization: The dried and pre-weighed films (BTA 40:60 or PCL-S MaterBi ZF03U/A) were sterilized by UV irradiation or hydrogen peroxide treatment as described by Wallhäuser (1984) and Belal (2003).

A copolyester made of 1, 4-butanediol, terephthalic acid and adipic acid in Fig. 1 was used to isolate degradative microorganisms.

Preparation of clear zone plates with BTA 40:60: For synthetic polyesters a suitable method to prepare turbid agar plates was described by Augusta *et al.* (1993) and Belal (2003). The polyester (0.25 g) was dissolved in 5 mL methylene chloride and the solution was then emulsified by sonication into 250 mL of MSV medium containing 1.5% (w/v⁻¹) agar-agar. The emulsion was then stirred continuously while heating

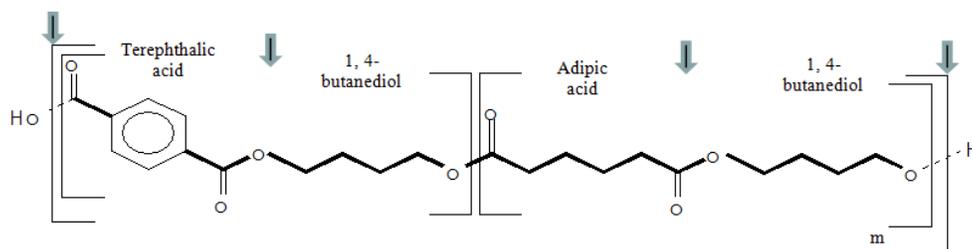


Fig. 1: Formula of the aliphatic-aromatic copolyester BTA 40:60 used for the screening of microorganisms with regard to their degradation abilities. The copolyester consists of 1, 4-butanediol, terephthalic acid and adipic acid

for at least 30 minutes to evaporate the solvent completely. The pH was adjusted to $\text{pH } 7 \pm 0.2$. The autoclaved medium resulted in homogenous opaque plates where it was poured into plates (9 cm in diameter) with 15 mL/plate.

Clear zone plates with natural polyester poly- β -hydroxybutyric acid (PHB): For clear zone tests (Augusta *et al.*, 1993; Belal, 2003), PHB powder was added to MSV medium at a final concentration of 0.1% (w/v) and the mixture was ultra-sonicated for five to 7 min. The medium was sterilized at 121°C and a pressure of 1 bar for 20 min because it has melting point 180°C . 15 mL medium were poured before cooling for each plate (9 cm in diameter).

Clear zone plates with (carboxymethyl cellulose) as natural polymer: MSV agar containing carboxymethyl cellulose (10 g/L) was used. The cultures were inoculated and incubated for 1-2 days at 55°C . The plates were treated and the clear zone was measured according to the method described by Peciulyte (2007) and Belal (2013a).

Media: Mineral Salt Vitamin medium (MSV) according to Belal (2003) was used for isolation of BTA-copolyester degrading microorganisms and evaluation of polyester degradation with pure isolates as well as TSB for actinomycetes and nutrient broth for other bacteria as well as PDA for fungi were used as a complex media.

Compost burial and isolation of BTA-degrading microorganisms: Polymer films (BTA40:60) of about 3.0×3.0 cm with initial weight of 0.21 g were buried at $\sim 50^\circ\text{C}$ up to $\sim 70^\circ\text{C}$ for one week in compost pile during thermophilic phase (containing from rice straw and animal manure in Department of Agricultural Botany (Agricultural Microbiology), Faculty of Agriculture, Kafrelsheikh University, Egypt). The films were hanged with net thread for easy follow up. Small residues debris from buried polymer films and net adhering compost were taken and dissolving 10 g in 90 mL MSV-medium supplemented with 0.1% w/v of suspension from polyesters (BTA 40:60). The culture

was incubated at 55°C and 160 rpm for one week. After one week, 10 mL of culture broths were transferred into fresh 90 mL mineral salt liquid medium containing 0.1% w/v of suspension from BTA40:60. This procedure was repeated three times. Dilutions series were prepared after the final time (after one week) from enrichment culture in glass tube containing 9 mL MSV liquid medium up to $1:10^6$. 100 μL from the three later dilutions from series were spreaded on emulsified MSV-agar containing 0.1% w/v of BTA 40:60 by using glass spreader. BTA-degrading microorganisms were selected on the basis of the hydrolysis (clear) zone surrounding the colonies as described by Augusta *et al.* (1993) and Belal (2003). Positive strains forming colonies surrounded by clear zones after incubation for 7 days at 55°C were isolated by picking the colonies using sterile tooth picks or needle inoculation and were further purified on complex media (Potato Dextrose agar for fungal isolates, TSB for filamentous bacteria as well as nutrient broth for bacteria). For bacterial strains the standard spatial streaking method on solid agar plates was used. Fungal isolates were purified by addition of ampicillin 800 mg/L to complex agar media (Potato Dextrose Agar). The cultures were identified based on the cultural, morphological, biochemical characteristic and 16S rDNA as described by Lechevalier and Lechevalier (1957), Rifai (1969), Crawford (1975), Domsch *et al.* (1980), Parry *et al.* (1983), Burgess *et al.* (1994), Miyadoh *et al.* (1987, 1989, 1990), Wang *et al.* (1996) and Zhang *et al.* (1998).

Degradation test with isolated strains:

Polyester depolymerization measured by clear zone formation: The potential of the isolates to depolymerize different polyesters was preliminary examined using the clear zone method (Augusta *et al.*, 1993; Belal, 2003). For the fungal isolates each agar plate containing BTA40:60 (0.1% w/v) was inoculated in the center with a disk (5 mm in diameter) from a complex medium (Potato Dextrose agar) with the fungal mycelium of a culture grown for 1 week at 55°C . For the bacterial strains the turbid MSV-agar plates containing the polyester (0.1% w/v) were inoculated with bacterial colonies from agar plates (TSB) or

nutrient cultures of the different strains with inoculation needles and incubated for 1 week at 55°C. The plates were incubated for 1 week at 55°C. The increase in clear zone diameters developing on the MSV-agar plates was followed up periodically and measured by slide gauge. Plates not inoculated with microorganisms were used as control. Experiments were made in triplicates.

Weight loss determination of polymer films on agar plates: As a quantitative measure for polyester depolymerization the determination of the weight loss of polymer films (70-85 mg) laid on agar plates and inoculated with the purified isolates. Three preweighted, sterile circular films (25 mm diameter, surface area assessable for degradation (one side = 4.91 cm²) of BTA 40:60 were placed on a MSV-agar plate and inoculated with 200 µL from fungal (10⁶ cfu/mL) and bacterial (10⁸ cfu/mL) suspension on surface of the polyester film.

The seed culture was prepared for fungal isolates in potato dextrose agar medium, incubated for 7 days at 55°C and in TSB-medium for filamentous bacteria as well as nutrient broth for bacterial isolates (30 mL medium in a 100 mL flask shaken at 150 rpm and 55°C for 3 days). The degradation time on the agar plates was for one week. Sterile controls incubated over the same period of time were performed. The weight loss of the polymer films was determined as described before and compared with the noninoculated control films.

Effect of pH and temperature on degradation of BTA-copolyester films by *Thermobifida fusca* and *Thermobispora bispora*: Three preweighted, sterile circular BTA40:60 films (25 mm diameter and surface area assessable for degradation 4.91 cm² and initial weight 70-85 mg) as a sole source of carbon were laid on a MSV-agar plate and inoculated with 200 µL from *T. fusca* or *T. bispora* containing 10⁸ cfu/mL (TSB liquid medium, 10⁸ cfu/mL; incubated at 55°C and 150 rpm for 3 days) suspension on surface of the polyester film. The experiments were carried out at pH 6, 7 and 8. The culture was incubated at 55°C for 1 week. To determine the optimum temperature, MSV- agar plate medium at pH7 was incubated at 45, 55 and 65°C for one week. The weight loss of the polymer films was determined as described before and compared with the noninoculated controls films.

Degradation of BTA 40:60, PCL-S MaterBi ZF03U/A and natural polymers (poly-β-hydroxybutyric acid (PHB) and carboxymethyl cellulose) by *T. fusca* or *T. bispora*: Three preweighted, sterile circular BTA 40:60 and PCL-S MaterBi ZF03U/A films (25 mm diameter and surface area assessable for degradation 4.91 cm² and initial weight 70-85 mg for BTA and 15-25 mg for PCL-S MaterBi ZF03U/A) as a sole source of carbon were laid

on a MSV- agar plate and inoculated with 200 µL from *T. fusca* or *T. bispora* containing 10⁸ cfu/mL (TSB liquid medium, 10⁸ cfu/mL; incubated at 55°C and 150 rpm for 3 days) suspension on surface of the polyester film. The experiments were carried out at pH7 and the culture was incubated at 55°C for one week. The weight loss of the polymer films was determined as described before and compared with the noninoculated controls films. I could not make films from PHB and carboxymethyl cellulose. *T. fusca* or *T. bispora* was tested for PHB and carboxymethyl cellulose degradation via clear zone formation a substrate spectrum as described above.

Effect of different carbon source on growth strains *T. fusca* or *T. bispora* and enzyme induction: Sterile circular polyester film (25 mm in diameter, surface area 9.82 cm² are 70-85 mg) and glucose (10 g/L), as sole of carbon source were added to 100 mL MSV liquid medium in 500 mL flasks and inoculated with 3 mL of a cell suspension of *T. fusca* or *T. bispora* (TSB liquid medium, 10⁸ cfu/mL; incubated at 55°C and 150 rpm for 3 days). TSB were used as complex medium and it was carried at the same conditions. Each flask for the media was inoculated with 3 mL of a cell suspension of *T. fusca* or *T. bispora* (TSB liquid medium 10⁸ cfu/mL; incubated at 55°C and 150 rpm for 3 days) and were incubated at 150 rpm and 55°C for one week. The activity of the enzyme was determined daily by filtration of the supernatant through (0.2 µm) and the supernatants were assayed for their enzymatic activity by measuring of clear zone on MSV plates containing BTA 40:60 as described above. The cultivations were compared with noninoculated controls. The bacterial cells were digested as described by Belal (2003) and the protein content was determined according to the method described by Lowry *et al.* (1951) using bovine serum albumin as a standard protein.

Substrate spectrum of crud enzyme different polyesters via clear zone formation: The culture broth was obtained by culturing of *T. bispora* or *T. fusca* strains with BTA 40:60 films. One sterile circular polyester film (25 mm in diameter, 70-85 mg and with surface area 9.82 cm²) was added to 100 mL MSV liquid medium in 250 mL flasks and inoculated with 3 mL of a cell suspension of *T. bispora* or *T. fusca* (TSB liquid medium 10⁸ cfu/mL; incubated at 55°C and 150 rpm for 3 days). The flasks were shaken at 150 rpm and 55°C for one week.

After incubation, the culture broth was centrifuged at 11400 rpm for 20 min and the supernatant was then filtrated through a sterile membrane filter (0.2 µm). The supernatant was concentrated by ammonium sulphate. Solid (NH₄)₂SO₄ was slowly added to the supernatant to a final concentration of 70% (w/v). The precipitated protein was collected by centrifugation 11400 rpm at 4°C) and Dialysis was carried out against phosphate buffer (pH7) for 12-24 h at 4°C in dialysis tubing's which were closed at both ends with clamps using. The

tubes were placed in a 100 fold volume of the respective buffer. The buffer was changed every 4-8 h. 100 μ L from the sterile and concentrated crude enzyme (with concentration of protein 0.994 or 0.985 mg/mL from *T. fusca* and *T. bispora*, respectively) was put in punched out holes (diameter 5 mm) in MSV-agar supplemented with 0.1% w/v of polyester suspensions (BTA 40:60 or PHB). The plates were incubated at 55°C for 12 h. Diameter of clear zone were measured by a slide gauge.

Degradation of BTA 40:60 films by *T. fusca* or *T. bispora* hydrolase: BTA 40:60-degrading activity was determined by measuring the weight loss of a BTA 40:60 films after incubation with the enzyme. Hydrolase activity was determined by incubating 0.5 mL of the supernatant (at a concentration 0.994 or 0.985 mg/mL from *T. fusca* or *T. bispora*, respectively) with one BTA 40:60 films ((25 mm in diameter, 70-85 mg and with surface area 9.82 cm²) in 100 mL Erlenmeyer flasks with magnetic bar. Final volume was adjusted to 50 mL MSV liquid medium (pH7) at 55°C for 24 h with agitation. The enzyme concentration was determined according to Lowry *et al.* (1951). The experiment was performed with 0.3 mg/mL chloramphenicol. After incubation, the weight loss of the polymer films was determined as described before and compared with control.

Measuring of BTA-hydrolase activity by clear zone method on tributyrin agar test: Tributyrin agar test was used to detect BTA-hydrolase activity. 100 μ L from the sterile and concentrated crude enzyme (with concentration of protein 0.985 or 0.994 mg/mL) was put in punched out holes (diameter 5 mm) in tributyrin agar and incubated for three days at 37°C. The plates were incubated at 55°C for 12 h. Diameter of clear zone were measured by a slide gauge. The presence of the lipase activity of the colonies was detected as opaque halos occurred around the colonies positive for the lipase activity (Haba *et al.*, 2000).

Effect of pH on the crude BTA-hydrolase activity (optimum pH): The pH optimum of the for the crude BTA hydrolase produced by *T. fusca* or *T. bispora* determined is described previously Kleeberg (1999) and Belal (2003).

Statistical analysis: Each experiment was performed in three replicates and the value is the mean of the three replicates. The Standard error was calculated with Microsoft Excel 2003

RESULTS AND DISCUSSION

Compost was used as microbial source at thermophile phase to isolate BTA40:60 degrading

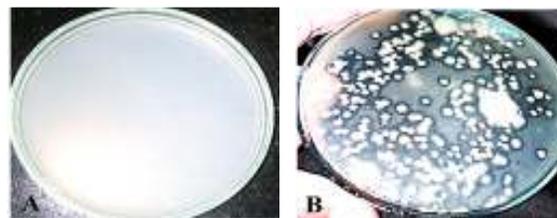


Fig. 2: Clear zone formation on BTA-MSV plates after 7 days at 55°C (inoculated from a 1 week old enrichment culture containing BTA40:60)

microorganisms in the present study. The results obtained in this study with BTA40:60 films buried in the compost at thermophilic phase under test revealed that about 100% of the added polymer was degraded BTA40:60 were degraded after one week.

Fifty microorganisms were isolated on emulsified MSV agar with 0.1% BTA40:60 nanoparticles by using clear zone formation on agar plates in Fig. 2. Ten BTA40:60 degrading microorganisms from the 50 isolated (comprising bacteria and fungi) as potential BTA hydrolase producers were selected and designated with code number (E30, E31, E32, E33, E34, E35, E36, E37, E38 and E39). A preliminary classification based on the morphology of the isolates revealed that the BTA 40:60-degrading microorganisms belong to the group of bacteria as well as to the group of fungi. Eight of 10 BTA 40:60 degrading microorganisms were bacteria. Five of 8 were gram-positive, non-motile, filamentous as well as 16S rDNA (Data not shown) and identified as *Thermobifida fusca* (E30), *Thermobispora bispora* (E31), *Thermoactinomyces* sp. (E32), *Saccharopolyspora* sp. (E33) and *Streptomyces* sp. (E34). Three of 8 bacterial isolates was gram -positive, motile, rods and spore former and identified as *Bacillus* sp. (E35, E36 and E37). Two of 10 BTA40:60 degrading microorganisms were fungi and identified as *Aspergillus fumigatus* (E38) and *Penicillium duponti* (E39). These results are in agreement with Kleeberg *et al.* (1998). The most widely used screening method for polyester depolymerizing organisms is the so called “clear zone” method (Augusta *et al.*, 1993; Belal, 2003). The extracellular hydrolyzing enzymes secreted by the target organism hydrolyze the suspended polyesters in the turbid agar medium into water soluble products thereby producing zones of clearance around the colony. The main advantage of this test is that it is generally fast, cheap and simple and allows the simultaneous performance of a great number of parallel tests.

Depending on the different physical properties of the used polyesters, the development of special emulsification methods for the different polyesters was necessary. For PHB, no special treatment was required and the polyester powder was directly mixed into the minimal agar medium prior to sterilization.

This test offers a number of advantages which described above. It can be used to obtain semiquantitative results by analyzing the growth of clear zones (Augusta *et al.*, 1993), but this technique was used only as preliminary test in the present work for the comparison between the isolates due to the reason, that fungi during their growth spread over the entire agar plate and thus, the clear zones formed cannot appropriately used even as a semi-quantitative measure for the degradation potential of the fungi (Belal, 2003). Clear zone formation was used as a sign for the biodegradation of the polyesters as a preliminary test at the different temperatures. Additionally, this indicates that there is no correlation between the growth and the degradation process at low temperatures (15 and 4°C) since the strain *Aspergillus fumigatus* (EB19) was grown at low temperatures on mineral salt medium with glucose and without clear zone formation on MSV plates containing BTA45:55 or PCL. It was of interest now to determine the degradation potential more quantitatively by the determination of the degradation rate by weight loss measurements with polymer films on agar plates (Belal, 2003).

With regard to the application of the isolated strains for improved degradation tests of synthetic polyesters and the difference in the growth behaviour of the isolated BTA40:60 degrading microorganisms, i.e. the estimation of weight loss for polymer films on agar plates was used. The degradation potential of all isolates was quantified (expressed as weight loss (mg) as degradation rate per surface area [mg/(week cm²)] for polymer films on agar plates after one week incubation at 55 °C.

Weight loss determination of polymer films on agar plates: The degradation potential of all isolates was quantified (expressed as weight loss (mg) and degradation rate per surface area [mg/ (week. cm²)] for polymer film on agar plates after one week incubation at 55°C.

Results in Fig. 3 showed that all isolates grew on mineral salts agar (MSV) plates containing polymer films (BTA 40:60) as a sole source of carbon. The obtained results give an overview of the number of isolated bacteria, actinomycetes and fungi and their abilities to degrade the aliphatic-aromatic copolyester BTA 40:60. A total of 10 strains of thermophilic microorganisms were isolated from buried BTA 40:60 films in compost at thermophilic phase. Among 8 bacterial isolates, which were all aerobically growing rods mainly with endospore formation, only three strains (*Bacillus* sp. E35, E36 and E37) were able to depolymerize BTA 40:60. Three of those strains exhibited degradation activities (weight losses of 3.1 to 5.5 mg/week. cm²). Two of 10 BTA 40:60 degrading films were fungi (*Aspergillus fumigatus* E38 and *Penicillium duponti* E39). The fungal strain were

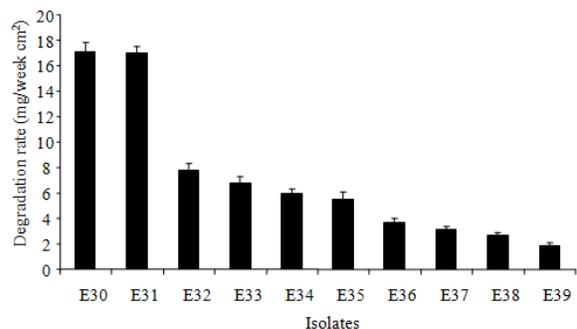


Fig. 3: Biodegradation of BTA40:60 films on MSV plates by the isolated microorganism

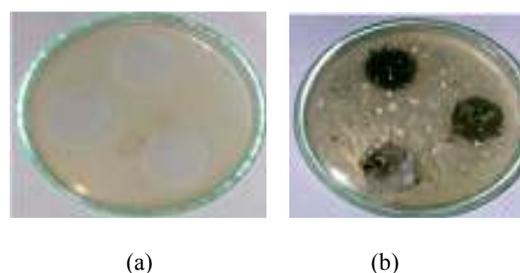


Fig. 4: Biodegradation of BTA 40:60 films on MSV agar by *Aspergillus fumigatus* E38

depolymerize BTA 40:60 films (weight losses of 1.83 to 2.65 mg/week. cm²) slower than the bacterial strains in Fig. 4. The degradation potential obtained for BTA copolyester by filamentous bacteria was higher than those of fungi. The general trend of biodegradability for BTA40:60 films with actinomycetes and bacteria > fungi.

Within the group of thermophilic actinomycetes, only 3 of 5 strains (*Thermoactinomyces* sp. E32, *Saccharopolyspora* sp. E33 and *Streptomyces* sp. E34) did show slow degradation activities (weight losses of 5.91 to 7.74 mg/week. cm²). Two of the most active actinomycetes, isolates *Thermobifida fusca* E30 and *Thermobispora bispora* E31, were identified taxonomically and used for further investigations. They degraded BTA films completely within 7 days (weight losses of 16.96 to 17.12 mg/week. cm²).

Obviously, thermophilic actinomycetes play an outstanding role in degrading the BTA copolyester with regard to both the number of microorganisms isolated and their degradation rates. The results were compared with those obtained with noninoculated films (noninoculated controls) incubated in the respective media. The noninoculated controls showed no weight loss.

Usually, enzymatic degradation of plastics is a surface erosion process, because enzymes are not able to penetrate the bulk polymer. Thus, the rate of weight loss can be directly used to measure the enzymatic cleavage of the polymer chains.

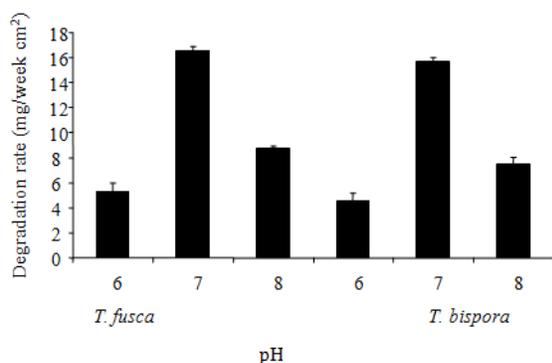


Fig. 5: Effect of pH on degradation of BTA 40:60 films on MSV plates by *T. bispora* and *T. fusca* by measuring degradation rate (mg/week. cm²)

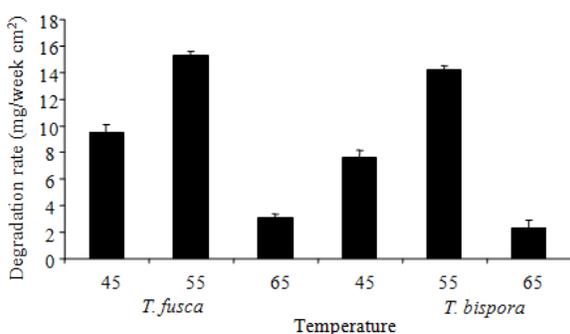


Fig. 6: Effect of temperature on degradation of BTA 40:60 films on MSV plates by *T. bispora* and *T. fusca* by measuring degradation rate (mg/week. cm²)

Our results are in agreement with previous findings reported by Kleeberg *et al.* (1998) who reported earlier that BTA 40:60 was degraded much faster than aliphatic polyesters like Bionolle, Bayer Tir 1874 and SP313 under thermophilic conditions by two strains from *Thermomonospora fusca*.

Obviously, filamentous bacteria play an outstanding role in degrading of the tested BTA-copolyester materials, since the majority of strains belong to this group. It is known that many genera of filamentous bacteria play an important role in degradation of anthropogenic substrates. Also for several natural polymers, like chitin, celluloses, starch and lignocelluloses fungi and actinomycetes are known to be involved in their degradation (Crawford and Sutherland, 1980; McCarthy and Cross, 1984; McCarthy, 1987; Kempf and Kutzner, 1988). From 10 screened isolates 3 BTA40:60 degrading stains fungal strains, namely *Thermobifida fusca* E30 and *Thermobispora bispora* E31 as filamentous bacteria were selected as they comprised the highest polyester depolymerization potentials and were used for further investigations.

Effect of pH and temperature on growth of *T. fusca* and *T. bispora* **Optimal pH:** The influence of the pH

of the BTA40:60 films degradation by *T. fusca* and *T. bispora* on MSV plates with surface area 4.91 cm² was also investigated at 55°C in Fig. 5. Generally, the optimum pH was 7 for *T. fusca* and *T. bispora*. The maximum degradation rate (mg/week. cm²) for BTA40:60 films were recorded at pH7 by the both strains. The degradation rate was 16.5 and 15.7 mg/week. cm² at pH7 by *T. fusca* and *T. bispora*, respectively. The degradation was investigated with different pH from 6.0 to 8.0. The results showed that the pH values were either higher or lower than 7, the rate of degradation was low, for the enzyme involved the degradation may lower activity in acid or alkaline conditions. This variation is very useful to use these isolates in degradation test in different environments at different pH. Therefore, it can expect that these isolates can tolerate the pH change during the degradation process thereby increase the degradation potential for these isolates.

Optimal temperature: Figure 6 illustrates that the influence of temperature on BTA 40:60 films degradation on MSV plates with surface area 4.91 cm² was also investigated by *T. fusca* and *T. bispora*. The degradation rate increased with increasing temperature by the both strains. The highest degradation with temperatures was higher than 45°C. The optimal temperature was 55°C. It must be admitted that weight loss measurements only indicate disintegration of the polymer films likely caused by cleavage of the polymer chains. Complete metabolism of the polymer material has to be investigated in additional tests. However, for polyesters, the first step in attacking the polymer chain is often the step which determines the degradability of such materials. The strains were able to degrade BTA 40:60 films at a low temperature. The experimental results were compared with those obtained from the noninoculated films (control), which showed no weight loss under the test conditions applied. Therefore, these strains were used for further studies under the optimum growth conditions.

Degradation of BTA 40:60, PCL-S MaterBi ZF03U/A, PHB and carboxymethyl cellulose by *T. fusca* or *T. bispora*: The obtained results in Table 1 showed that substrate spectrum for the selected polyester degrading strains characterized by degradation rate (mg/week.cm²) for the synthetic polyester and clear zone formation for natural polymers (PHB and carboxymethyl cellulose) on MSV-agar. The selected BTA 40:60 degrading strains were tested with PCL-S MaterBi ZF03U/A as another synthetic aliphatic polyester by determination of degradation rate and PHB and carboxymethyl cellulose as natural polymers by clear zone formation. The experiments were carried out at 55°C after 1 week with these strains. The results in

Table 1: Degradation of BTA 40:60, PCL-S MaterBi ZF03U/A, PHB and carboxymethyl cellulose by *T. fusca* or *T. bispora*

Strains	Degradation rate (mg/week.cm ²)		Clear zone formation (mm)	
	BTA 40:60	PCL-S MaterBi ZF03U/A	PHB	Carboxymethyl cellulose
<i>T. fusca</i>	15.3	5.1	25	52
<i>T. bispora</i>	14.7	2.82	23	48
Control	0	0.3	0	0

Table 1 show that, as expected, all strains can degrade BTA copolyester and PCL-S MaterBi ZF03U/A as synthetic polyesters under investigation. The both strains were able to attack PHB and carboxymethyl cellulose as natural polyesters. This shows that especially actinomycetes are versatile in their ability to degrade the polymers under investigation. The results in Table 1 summarize the differences of weight loss for polymer films. It is not expected, the general trend of the biodegradability was BTA40:60>PCL-S MaterBi ZF03U/A and this may be due to small the initial weight of PCL-S MaterBi ZF03U/A films in case use of *T. fusca*. On the other, *T. bispora* was not able to degrade starch and lead to reduce the PCL-S MaterBi ZF03U/A degradability by *T. bispora*. Additionally, the incubation time was determined after one time (one week). The experimental results were compared with the noninoculated (control), which showed no weight loss for BTA copolyester but it was loss with 0.3 mg/week.cm² in PCL-S MaterBi ZF03U/A films.

Belal (2003) isolated mesophilic actinomycetes and fungal strains degrades the synthetic polyester including BTA-copolyester (BTA 45:55 (Ecoflex) and BTA 40:60 PCL, SP 4/6) and PHBV as natural copolyester and these strains was identified as *Microbispora rosea* subsp. *rosae* (E11, Act3), *Streptomyces thermocarboxydus* (Act23), *Fusarium solani* (EB10), *Aspergillus fumigatus* (EB19). He isolated also mesophilic fungal strains degrades only the synthetic polyester including BTA-copolyester (BTA 45:55 (Ecoflex) and BTA 40:60 PCL, SP 4/6) and these strains was identified as *Arthrotrichum amerospora* (EB1), *Cladosporium herbarum* (EB2T), *Acremonium strictum* (EB14). The question has to be posed, what is the reason for the different substrate specificities. It has been reported in the literature, that at least different enzyme systems are involved in the aerobic degradation of the different polyesters. These different kinds of enzymes are PHB deplomyerases, lipases and cutinase. Aerobic PHB depolymerases show no significant lipase activity or attack synthetic polyesters.

The ability of individual microbial strains to degrade the synthetic and natural polyesters was investigated earlier by many authors. Pranamuda *et al.* (1995) showed that actinomycete strain HAT-6 can degrade Polyhydroxybutyrate, Polycaprolactone and Bionolle. Mergaert and Swings (1996) isolated 171 polymer-degrading isolates, from these isolates actinomycetes and fungal isolates were able to degrade for polyhydroxybutyrate, Bionolle and polycaprolactone. The obtained results are in agreement

with Kleeberg *et al.* (1998). Aerobic PHB depolymerases show no significant lipase activity or attack synthetic polyesters (Jaeger *et al.*, 1995). However, several lipases hydrolyze synthetic polyesters such as PCL, but on the other hand, cannot hydrolyze PHB (Pranumuda *et al.*, 1995). The third group of enzymes, cutinases are serine hydrolases for primary alcohol esters (Kazlauskas and Bornscheuer, 1998; Murphy *et al.*, 1996) which depolmerize cutin specifically and PCL probably due to structural similarities between its depolymerization products and those another structurally similar to cutin. Therefore, the behaviour of the degrading microorganisms in the both classes shown above can be explained as follows: In the first group, where the organisms are specialized to degrade the synthetic polyesters the organisms produce exclusively lipase or cutinase enzymes. On the other hand the organisms which degrade synthetic and natural polyesters as well, probably produce at least two different enzymes, which are depolymerase and either lipase or cutinase.

Regulation of BTA 40:60 hydrolase production (constitutive or inductive enzyme) from *T. bispora* and *T. fusca*:

According to Schlegel (1992) most enzymes systems involved in substrate degradation are inductive enzymes. Therefore it is of interest to know, if the BTA 40:60 degrading enzyme system is constitutively secreted or induced by the presence of BTA 40:60 or other carbon source. Furthermore it is of interest, if the enzyme is inducible, what are the substances inducing the enzyme activity? A series of experiments were carried out to study the growth behaviour for *T. bispora* and *T. fusca* in a mineral salts vitamin medium supplemented with BTA 40:60 films (one film /flask in 70-85 mg weight), glucose, (10 g/L) as a sole carbon source and compare it with the growth on TSB as complex media.

Figure 7A shows that the cell growth (concentration of intracellular protein) was high on TSB, followed by TSB+Glucose, TSB+Glucose+BTA 40:60 films, MSV+glucose, MSV+glucose+BTA 40:60 films, MSV+BTA 40:60 films and later on MSV without carbon source. The growth was slowly on BTA copolyester in comparison with the other cultivations. When each strain was cultivated on glucose plus BTA 40:60 in the same media, the cell growth was low in comparison to the culture in mineral salt medium supplemented only with glucose. This is probably due to a competitive inhibition for the two substrates.

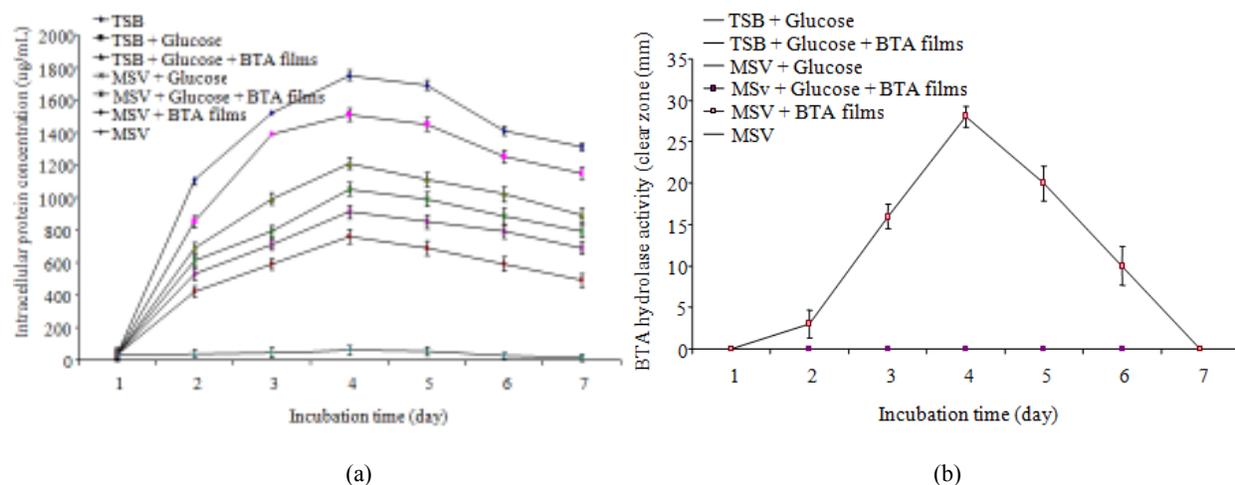


Fig. 7: Effect of BTA40:60 films, glucose and TSB as complex medium on growth of *T. fusca* and BTA40:60 hydrolase induction where Fig. 7A: Growth curve of strain *T. fusca* on TSB and MSV supplemented with different carbon sources and Fig. 7B: BTA40:60-hydrolase activity on the different carbon sources

Figure 7B illustrates that the extracellular BTA hydrolase formation started when the organism grew on strain *T. fusca* and *T. bispora* with BTA40:60 films in mineral salt vitamin medium as carbon sources. The results demonstrated that, enzyme formation in submerged culture started in the second day from the cultivation and after that the activity of the enzyme decreased at the end of the stationary phase. The activity was detected clear zone formation. The results demonstrated that a maximum hydrolase activity was obtained when BTA40:60 films were used as substrate in the fourth day. On the other hand in TSB as a complex media, TSB+Glucose, TSB+BTA 40:60 films, TSB+Glucose+BTA 40:60 films, MSV+Glucose, MSV+ Glucose+BTA40:60 (films), MSV+BTA40:60 films and MSV without any carbon source repressed BTA40:60 production. Enzyme secretion is not induced despite the media generated a good cell growth. Similar results were obtained during cultivation of *T. bispora* on the same carbon sources to know if the previous carbon sources induce BTA40:60 hydrolase or not (Data not shown).

The induction of hydrolytic enzymes by insoluble polymers raises the question, how such a polymer passes through the outer cell membranes. Large structural materials, such as macromolecules, cannot pass through outer cell membranes. Therefore, the presence of polymers induces or enhances the microbial production of enzymes which are excreted into the environment and are capable of cleaving specific bonds in the polymer chain, making them available for the enzymatic system on the surface of the polymeric material. Thus, the solid polymer is destroyed layer by layer and short chain and water-soluble intermediates and monomers are generated, which can be assimilated into the cells. According to Lin and Kolattukudy (1980) microorganisms secrete continuously low amounts of

various extracellular hydrolyzing enzymes into their surroundings. The depolymerization products thereby produced are taken up into the cell, where they can induce the synthesis of appropriate amounts of the required or favorable hydrolyzing enzyme. The authors supported their hypothesis with investigations of the phytopathogenic fungus *Fusarium solani f. pisi*. The induction of cutinase by this fungal strain did not only take place in the presence of cutin in the medium, but also at low levels of substrate hydrolyzate which would consequently be generated and then enter the cells to induce the synthesis of the enzyme. Cutinase activity is induced in the supernatant of *Fusarium solani f. pisi* and other fungal phytopathogenic fungus cultures in media containing cutin as a carbon source. The repression of cutinase formation by glucose could be proven. Murphy *et al.* (1996) found also that the presence of glucose in the PCL agar medium repressed the degradation of PCL. Oda *et al.* (1995) showed also that the presence of either soluble starch, lactose or glucose repressed the synthesis of PHB and PCL depolymerase by *Paecilomyces lilacinus*. In the present work we found also that the PCL-hydrolase was induced in the culture supernatant with PCL or BTA 45:55 (Ecoflex) as substrates, but was not induced on glucose or GYM media. These results are in agreement with my previous findings and other investigators while secretion of PCL-hydrolase was only induced in the culture supernatant with PCL as an aliphatic homopolyester or BTA 45:55 (Ecoflex) as a copolyester as substrates but was not induced on glucose or GYM as complex medium with *Microbispora rosea* subsp. *rosea* (Lin and Kolattukudy, 1980; Murphy *et al.*, 1996; Belal, 2003).

BTA-hydrolase production from strains *T. bispora* and *T. fusca* in submerged culture with BTA40:60 (nanoparticles or films): As expected, the growth was

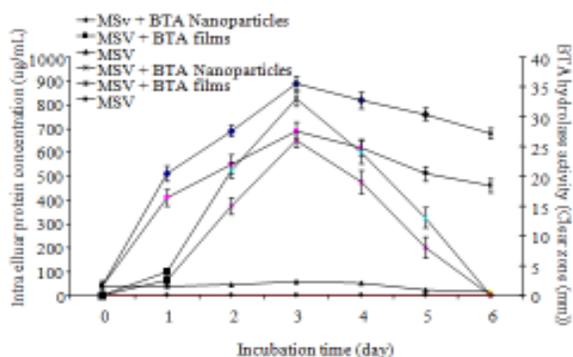


Fig. 8: Effect of surface area on BTA40:60 hydrolase production

slowly on BTA40:60 films in comparison with BTA40:60 nanoparticles in Fig. 8. The reduced growth is due to the smaller surface area of the films to be attacked by the microorganisms. In contrast, the organism grew much faster on nanoparticles than on the films due to their small size and larger surface area.

Figure 8 illustrates that the extracellular BTA40:60 hydrolase formation started when the organism grew on BTA40:60 nanoparticles. The results demonstrated that, enzyme formation in submerged culture started in the second day from the cultivation and after that the activity of the enzyme decreased at the end of the stationary phase. The enzyme activity disappeared, when all BTA40:60 were consumed by the organism. The BTA40:60 hydrolase activities were detected by clear zone formation. No BTA40:60 could be detected in the treatments with strain *T. fusca*.

The results shown in Fig. 8 demonstrated that the enzyme formation with the BTA40:60 films started in the second day and the films decomposed into fragments after 3 days. The activity of the enzyme decreased then at the end of the stationary phase. The activity disappeared, when the BTA40:60 films were degraded completely. The activity was detected by determination of clear zone. BTA40:60 films were degraded completely after 3-4 days in liquid mineral salts medium but the films were degraded completely at the same conditions after one week with the same strain on solid agar medium. This difference in the degradation rate may be explained as described above.

The same trend in effect of surface area on production of BTA40:60 *T. bispora* hydrolase (Data not shown). Similar results were obtained during cultivation of *T. bispora* on BTA40:60 films or nanoparticles to know the effect of surface area on growth of *T. bispora* and production of BTA40:60 *T. bispora* hydrolase (Data not shown).

Degradation of BTA 40:60 films with BTA hydrolase from *T. bispora* and *T. fusca*: BTA hydrolase from *T. fusca* and BTA hydrolase from *T. bispora* exhibited BTA 40:60-degrading activity at pH

Table 2: Substrate specificities of BTA 40:60 -hydrolase on MSV-agar plates supplemented with synthetic or natural polymers (0.1%) after 12 h at 55 °C

Polyester	Clear zone (diameter mm)	
	<i>T. bispora</i>	<i>T. fusca</i>
1-Synthetic polymers		
BTA40:60	38	36
2- Natural polymers		
PHB	0	0
Carboxymethyl cellulose	0	0

Table 3: Substrate specificities of BTA 40:60 -hydrolase on tributyrin agar plates after 12 h at 37°C

Strains	Clear zone (diameter mm)
<i>T. bispora</i>	45
<i>T. fusca</i>	40
Control	0

7 and 55°C after incubation for 24 h. The obtained results showed that, the degradation rate with BTA hydrolase from *T. fusca* was 3.67 mg/day. cm² (0.153 mg/h.cm²) and it with BTA hydrolase *T. bispora* was 3.5 mg/day.cm² (0.144 mg/h.cm²). The experimental results were compared with the noninoculated (control), which showed no weight loss for BTA copolyester. BTA hydrolase has been reported to degrade BTA copolyester (Kleeberg, 1999).

Determination of substrate specificities of the crude BTA 40:60- hydrolase via estimation of diameter clear zone on MSV-agar containing the polyesters (0.1% w/v) at 55°C: Degradation of BTA 40:60 as synthetic polyester and PHB, starch as well as carboxymethyl cellulose as natural polyesters was investigated with *T. fusca* and *T. bispora* on agar plates and with weight loss determination of polymer films on agar plates. The question remained open whether the organism produced different hydrolytic enzymes for individual polyester structures or whether one substrate specificity has the BTA 40:60 hydrolysing enzyme sufficiently broadly to degrade all structures. The results in Table 2 show that the BTA40:60 hydrolase was able to form clear zones with BTA 40:60 as synthetic polyester including. No hydrolytic activity was observed for PHB and carboxymethyl cellulose as natural polyesters by the BTA 40:60-hydrolase. However, PHB and carboxymethyl cellulose were degraded by strain *T. fusca* and *T. bispora* on agar plates via clear zone formation. This proves the ability of strains *T. fusca* and *T. bispora* to produce different hydrolases, PHB-depolymerase, BTA-hydrolase and cellulase. PCL (poly (ε-caprolactone) as aliphatic homopolyester is degraded by lipases and esterase's (Tokiwa and Suzuki, 1977) and a PHB depolymerase did not hydrolyze PCL (Jaeger *et al.*, 1995). Therefore, probably the PCL-hydrolase is a cutinase or lipase but no PHB depolymerase. These results are in agreement in my previous findings (Belal, 2003).

Substrate spectrum of the crude BTA-hydrolase concerning Tributyrin as fatty acids (triglyceride): The results in Table 3 show that the BTA-hydrolase from *T. fusca* or *T. bispora* was able to form clear zone

with tributyrin. The natural substrates of lipase are triglyceride of long-chain fatty acids. These triglyceride are insoluble in water and lipases are characterized by the ability to rapidly catalyse the hydrolysis of ester bonds at the interface between the insoluble substrate phase and the aqueous phase in which the enzyme is soluble. A number of different substrates have been used for the assay of lipases. Tributyrin agar is a differential medium that tests the ability of an organism to produce an exoenzyme, called lipase that hydrolyzes tributyrin oil. Lipases break down lipids (fats Lipase allows the organisms that produce it to break down lipids into smaller fragments. Triglycerides are composed of glycerol and three fatty acids. These get broken apart and may be converted into a variety of end-products that can be used by the cell in energy production or other processes. Tributyrin oil forms an opaque suspension in the agar. When an organism produces lipase and breaks down the tributyrin, a clear halo surrounds the areas where the lipase-producing organism has grown. All lipases and cutinase hydrolysed triglyceride (Jaeger *et al.*, 1995). Lipases are known to be able to hydrolyze synthetic polyesters such as PCL, while PHB depolymerases, which are enzymes designed by nature to cleave ester bonds in natural PHB, usually are not able to hydrolyze synthetic polyesters. Therefore, probably the BTA 40:60-hydrolase is cutinase or a lipase but not a PHB depolymerase. The presence of the lipase activity of the colonies was detected as opaque halos occurred around the colonies positive for the lipase activity (Haba *et al.*, 2000). These results are in agreement in my previous findings (Belal, 2003).

Effect of pH on the crude PCL-hydrolase activity (optimum pH): For the determination of the optimum pH of the BTA-hydrolase activity different buffer systems were used. For a pH range of 4 to 7 a 150 mM citrate buffer was used. The pH values of 5.5 to 8 were prepared with 20 mM phosphate buffer and the remaining pH value from 5.5 to 8 were obtained using 100 mM phosphate buffer, BTA hydrolase was used at a protein concentration 0.994 or 0.985 mg/mL from *T. fusca* or *T. bispora*, respectively. The activity for BTA hydrolase at different pH was measured by using clear zone technique. The optimum pH for BTA hydrolase was at pH7 with both two phosphate buffer (20 and 100 mM phosphate buffer) in Fig. 9. Yet, a second optimum pH appeared at a pH 6 with citrate buffer (150 mM). It is known also that the optimum pH for growth the strain *T. fusca* or *T. bispora* on mineral salt medium with glucose was pH7. This buffer system was used earlier by Kleeberg (1999) and Belal (2003) to study the effect of pH on the activity for hydrolase from *Thermomospora fusca* and PCL hydrolase from or *Microbispora rosea subsp. rosae*. The author found that pH optimum for hydrolase from *T. fusca* was 6.5 with both phosphate buffer (20 and 100 mM) and the second optimum pH was at 6 with citrate buffer (150 mM). Belal (2003) found that pH optimum for PCL hydrolase

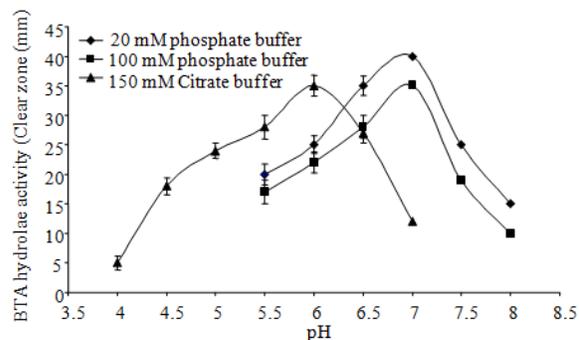


Fig. 9: Effect of different pH on activity of BTA- hydrolase from *T. fusca*

from *M. rosea subsp. rosae* was 7 with both phosphate buffer (20 and 100 mM) and the second optimum pH was at 6 with citrate buffer (150 mM). This buffer system was used earlier by Kleeberg (1999) and Belal (2003) to study the effect of pH on the activity for hydrolase from *Thermomospora fusca* or *Microbispora rosea subsp. rosae*. Similar results exhibited that that pH optimum for BTA40:60 hydrolase from *T. bispora* was similar to pH optimum of BTA40:60 hydrolase from *T. fusca* (Data not shown).

CONCLUSION

The results of the present study concluded that the aromatic-aliphatic copolyester (BTA), PCL-S MaterBi ZF03U/A and as well as the natural polyester poly (hydroxybutyrate-co-hydroxyvalerate) (PHB) and carboxymethyl cellulose was investigated under thermophilic conditions and that the biological agent was mainly actinomycetes such as *Thermobifida fusca* and *Thermobispora bispora*. The degradation was evaluated with different methods including clear zone, weight loss and all results prove the degradability of the polyesters under test. The results from this study can be applied to a highly effective and environmental biodegradable plastics production. However, this study is a pioneer in applying *Thermobispora bispora* to BTA40:60 film degradation. The degradation rate for BTA films within 7 days was 17.12 and 16.96 mg/week.cm² by *T. fusca* and *T. bispora*, respectively. The results indicate two variables; pH and temperature (°C) have significant effects on BTA degradation. The optimum condition is obtained as: pH 7 and 55°C. In addition, the both strains degraded BTA40:60 films completely within one week on agar plates.

The both strains exhibited a wider substrate spectrum as they are able to degrade synthetic polyester such as BTA40:60, PCL-S MaterBi ZF03U/A and as well as natural polyesters such as PHB and carboxymethyl cellulose.

It was shown that the extracellular hydrolyase activity was induced by the presence of BTA-copolyester, while the presence of additional carbon

sources such as glucose or a complex medium suppressed enzyme formation. The concentrated crude BTA-hydrolase was active towards tributyrin as triglycerides and a wide range of synthetic polyesters (BTA copolyester). In contrast the enzyme was not capable to depolymerize the natural polyester PHB and carboxymethyl cellulose, although the organism itself degraded both types of polyesters (synthetic and natural polyesters). The finding indicates the presence of at least three different extracellular enzymes for this strain, probably a PHB depolymerase, a lipase and cellulase.

The obtained results showed that, the degradation rate with BTA hydrolase from *T. fusca* was 3.67 mg/day. cm² (0.153 mg/h. cm²) and it with BTA hydrolase *T. bispora* was 3.5 mg/day.cm² (0.144 mg/h. cm²). The general trend of bioconversion of different BTA40:60 films with BTA hydrolase was more than the general trend of bioconversion of 40:60 films by *T. fusca* and *T. bispora*. The results obtained in this study provide useful information about the degradation behavior of polyester materials under thermophilic conditions, which is importance for recycling of biodegradable plastics in composting process. Further study is needed to detect the genome sequences as well as purification of BTA hydrolases and using their in different industrial applications.

ACKNOWLEDGMENT

Thanks to the soul of Prof. Deckwer and to Dr. Müller, Gesellschaft fuer Biotechnologische Forschungen (GBF, Braunschweig, Germany) for supplying me with the plastics materials.

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