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An Experimental Investigation of PAH Emissions from a Heavy Duty Diesel Engine Fuelled with Biodiesel and its Blend

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Abstract. For the comparison of emissions of polycyclic aromatic hydrocarbons (PAHs) from diesel, biodiesel and its 20\% blend with diesel, and their carcinogenic potencies, an experimental study has been conducted on a turbocharged, intercooled and direct injection diesel engine. Total PAHs (solid and gas) from diesel, B20 and B100 at low load were more than those at high loads. Total PAH emissions from the test fuels at the rated speed were more than those at maximum torque speed. Benzo[a]pyrene (BaP) brake specific emission of biodiesel is less than that of diesel. LMW-PAH emissions for the test fuels are all higher than those of MMW and HMW PAH. Biodiesel and B20 reduce both the total Benzo[a]pyrene equivalent concentration (BaPeq) and the total mean–PAHs as compared to commercial diesel fuel. BSFC of the engine increased but its brake power decreased in the cases of B20 and biodiesel.

Keywords: diesel engines, biodiesel, polycyclic aromatic hydrocarbons, carcinogenic potencies

Introduction

Diesel engines due to their high fuel efficiency, out put power and fuel economy are widely used in heavy duty trucks, buses, generators, construction and agricultural machinery, in the face of the dwindling sources of conventional fossil fuels, their ever increasing demand and prices and stringent emission regulations. Among a number of alternative fuels like methanol, ethanol, LPG, LNG, CNG and vegetable oils, biodiesel consisting of alkyl monoesters of fatty acids from vegetable oils or animal fats can be used in unmodified diesel engines in pure or blended forms (Meher \textit{et al}., 2006; Graboski and McCormick, 1998). It is a non-toxic, eco-friendly and biodegradable fuel (Lapinskiene \textit{et al}., 2006). Use of biodiesel as an alternative fuel reduces the regulated air pollutants, including particulate matter, HC, CO and SO\(_{2}\) (Labeckas and Slavinskas, 2006; Sinha and Agarwal, 2005; Usta, 2005; Senda \textit{et al}., 2004; Turrio-Baldassarri \textit{et al}., 2004; Monyem and Van Gerpen, 2001). Global use of biodiesel can curtail green house gas emissions as compared to mineral diesel (Gerpan, 2006; Carraretto \textit{et al}., 2004; Tan \textit{et al}., 2004; Peterson and Hustruid, 1998). It has higher cetane number, ultra-low sulphur concentration, higher flash point, high oxygen content and improved lubricating efficiency (Ebiura \textit{et al}., 2005; Agarwal \textit{et al}., 2003; Fukuda \textit{et al}., 2001). Biodiesel has less adverse effect on human health as compared to diesel (Schroder \textit{et al}., 1999) and mutagenicity of biodiesel particulate emissions is much lower than that of petroleum-based diesel fuel (McDonald \textit{et al}., 1995). To encourage the use of biodiesel fuel, Austria, Germany and United States, Governments have announced tax benefits for the people (Raneses \textit{et al}., 1999; Krawczyk, 1996).

Although biodiesel has widely been investigated as an alternative fuel in diesel engines for performance, regulated and somewhat unregulated emissions, however polycyclic aromatic hydrocarbons (PAHs) and their carcinogenic potencies still need to be addressed comprehensively. This study is an effort to determine PAHs and their corresponding carcinogenic potencies from the exhaust of a diesel engine alternately fuelled with biodiesel and its 20\% blend with commercial diesel fuel.

PAHs are formed by incomplete combustion or high temperature pyrolytic process involving organic matter (Khalli et al., 1995). PAHs are semi volatile substances at atmospheric conditions and occur both in vapour-phase and as attached to particles depending on vapour pressure of each PAH component (Basheer \textit{et al}., 2003). Lighter PAHs are in vapour phase, while those having four or more rings are found mainly adsorbed in particulate material (Park \textit{et al}., 2002). Although lighter PAHs have weaker carcinogenic properties, they are the most abundant in the urban atmosphere and react with other pollutants to form more toxic derivatives (Ho \textit{et al}., 2002). PAHs specially benzo (a) pyrene injure the respiratory and immune system, and is responsible for cell mutation and can cause skin and lung cancers (Grevenynghe \textit{et al}., 2003; Yousef \textit{et al}., 2002). PAHs are mainly contributed by the mobile sources like diesel and gasoline engines; the contribution of total PAHs from mobile sources to ambient air is 91.8\% (Yang \textit{et al}., 1999).
Recycling of secondary raw materials is an important part of the industries in developing countries like Pakistan. Silver is a valuable natural resource of finite supply; it is used as a component of various products. The discharged silver remains a pollutant of concern due to its aquatic toxicity and is subject to control by both hazardous waste and water quality regulatory programmes. Wastewashings containing silver at a concentration greater than 5 mg/l are regulated as hazardous waste (Paul, 1997) and cannot be discharged without first being rendered non-hazardous. Silver has monetary value as a recovered commodity as well. Harper and Siegel (2003) studied two different types of processes, electrolytic plating units alone and electrolytic plating units in combination with metallic replacement backup units and using them, they described the recovery of silver, down to concentrations of less than 5 mg/l.

Major source of recoverable silver is photo processing activity wherein the metal appears in different forms depending on the type of process. The photographic processing industry has four options in silver recovery i.e., electrolytic plating, metallic replacement, ion exchange and chemical precipitation. Chemical precipitation and recovery of silver by gravimetric methods is an effective technology. The precipitation process converts soluble metal compounds into relatively insoluble sulphide compounds through the addition of precipitating agents, such as sodium sulphide (Na₂S). Over a broad pH range, sulphides (S²⁻, HS⁻) are extremely reactive with heavy metal ions. Sulphide precipitation can be operated over a wide range of pH, typically from pH 2 to 12. Metal-sulphide precipitates are less amphoteric than the corresponding metal-hydroxides and, therefore, less likely to resolubilize because of changes in pH.

Extraction of silver from industrial wash waters of film-printing plants has been the subject of interest of many investigators. Syed et al. (2002) obtained a high yield of silver on heating the films with oxalic acid solution to boiling temperature to separate the inorganic components from the polymer substrate. Lanzano et al. (2006) studied one year anthropogenic stocks and flows of silver as it progresses from extraction to final disposal. They concluded that in total 62% of all discarded silver is recycled and 38% is sent to landfills. Rivera et al. (2007) studied silver precipitation in elemental form in the S₂O₃²⁻ - S₂O₄²⁻ system and recorded recovery of more than 99%. Arsalan and Sayiner (2008) followed the process of thiosulphate leaching of precious metals and recovered 99.57% Au and 95.87% Ag at optimized conditions. Zhouxiang et al. (2008) reported total silver recovery from the spent fixing bath and waste X-ray films as 98.0% and 95.8%, respectively.

In the present investigation, X-ray washings, were treated with activated carbon under certain conditions prior to its precipitation as silver sulphide in a bid to separate the inorganic compounds from gelatin and polymer compounds to gain the maximum recovery of silver. Possibilities of application of the developed procedure for processing of secondary raw materials containing silver, at large scale, were also explored.

Key words: silver recovery, activated carbon, X-ray washings
Comparative Study of Quality Changes in Okra

*Abelmoschus esculentus* (L) Moench Stored at Different Relative Humidities

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Abstract. Okra (*Abelmoschus esculentus* L) pods were stored at the relative humidity of 90% and 100% for up to 10 days. The moisture content, crude fibre, and protein, fat, viscosity, hydrolysable and condensed tannin, total phenol, vitamin C and reducing power of the okra were determined on alternate days. Okra pods stored at 100%RH experienced the least percentage loss in all the determined parameters though the loss of antinutrient was lower at this relative humidity.

Keywords: relative humidity, antioxidant, okra, storage conditions

Introduction

Okra (*Abelmoschus esculentus* (L) Moench) is a tall annual dicotyledonous plant widely grown as vegetable crop in the tropics and subtropics and also in the warmer temperate areas (Kochhar, 1986). It has many different cultivars, varying in many respects. (Tindall, 1986). Okra is traditionally grown in African regions, the most important production regions being Ghana, Burkinafaso and Nigeria (De Lannoy, 2001).

Fresh and green tender fruits of okra are used as vegetable, whereas its mucilage has medicinal applications. Okra has industrial applications as well and is used in confectionary (Siemonsma and Kouame, 2004; Shalau, 2002; Kochhar, 1986).

Okra is a powerhouse of valuable nutrients. Nearly half of its fibre is soluble and half is insoluble which helps to keep the intestinal tract healthy (Shalau, 2002). The fibres from stem and mature pods have a number of uses in papermaking, in textile and other industries. (Siemonsma and Kouame, 2004; Shalau, 2002; Kochhar, 1986). In Nigeria, fresh okra is preferred to dried okra by the majority of the people and as such its consumption is highest in the rainy season when production is at its highest. The sites of okra production are always very far from the market, therefore, post-harvest deterioration of fresh okra results in loss of produce due to poor storage and transportation, to long distance, optimum relative humidity might be needed for storage life extension. The aim of this research work is to investigate the quality changes in Akure indigenous okra when stored at the relative humidities (RH) of 90% and 100%. The qualities assessed are nutrients, antinutrients, antioxidants and viscosity.

Materials and Methods

Okra plant (*Abelmoschus esculentus* L. Moench) used in this study was of indigenous origin in Akure, Nigeria. Plants were grown on a fallow land of 5 years, measuring 14 m×14 m, located in a farm in Ifon in Ose local Government of Ondo State, Nigeria. The experiment was laid out in randomized complete block design (RCBD). Each experimental unit was planted on the side of ridges. Spacing was 0.9 m between and 0.45 m within rows. Three seeds were planted per hole and thinned to one per stand, two weeks after planting (WAP), giving a plant population of about 24,690 plants/hectare. Each potential okra pod was tagged on the day, the flower dropped and pods free of apparent mechanical injuries, insect damage or diseases were harvested using knife on the 8th day after the flower had dropped.

Pods were randomly divided into two lots and stored at 90% and 100% relative humidities at the temperature of 10 °C ± 2 °C for 10 days. The temperature of storage rooms were controlled using a single point thermostat. Relative humidity inside the storage room was manually measured daily using wet bulb/dry bulb hygrometer.
Introduction

During the last three decades, Gulf’s agricultural production has dramatically increased. (Talukder and Kaakeh, 2006; Thacker et al., 2000) which is associated with different factors including the increased use of agrochemicals, especially pesticides for crop protection (Tivy, 1991). Since 1960, the value of pesticide imports to Oman has increased more than 10 fold (FAO, 2003). Around 835 pesticides have been registered in the UAE, and among them, the insecticides have the greatest share (49.8%) followed by fungicides (22%). The average amount of pesticides used in the UAE has been 9.86 l or kg per hectare per year (Kaakeh et al., 2004). This increased use of different pesticides in Gulf countries is a cause for serious concerns. Hazardous effects of pesticide residues in plants, soil, water, food, environment, humans and domestic animals and on beneficial organisms including pollinating honeybees, predators, parasites, fishes, birds and other wild animals are very serious issues world wide (Perry et al., 1998; Edwards, 1987). Pesticides may also cause resistance and resurgence problems in target pest populations, resulting in use of their higher doses at greater frequencies in the farms (Talukder and Kaakeh, 2006).

The use of pesticides in vegetable production in Oman is a regular practice. In Northern Oman all agricultural farms use different types of pesticides for the protection of crops (Kaakeh et al., 2007; Thacker et al., 2000); 95% of the farms use insecticides, 60%, fungicides and 20%, herbicides. Regular use of pesticides in crop production might be associated with pesticide residue problems. Three out of 11 selected pesticides, widely used in the UAE, have the potential to leach to groundwater due to their solubility that exceeds the US EPA threshold values (Kaakeh et al., 2004). Therefore, over the years, contamination of soil and ground water with the pesticides has been a major concern. The present investigation was designed to determine pesticide residue levels in agricultural soils of Omani and UAE vegetable farms, so as to estimate the extent of soil pollution caused by the use of pesticides under local environmental conditions. It may consequently aid in developing better management strategies.

Materials and Methods

Sampling techniques. In Oman, twenty farms located at different sites were selected from the Al-Batinah region, from which soil samples were collected randomly from two locations in each farm at two different depths (0-15 cm and 15-30 cm). In the UAE, twenty farms representing four agricultural regions of Al-Ain (five farms per region) were selected for the present study and soil samples were collected in a similar way. Soil samples were separately sieved to exclude foreign materials and the samples were stored in previously marked plastic bags which were transferred to Central Laboratory Unit, UAE University for analysis. The types of pesticides applied in all of these surveyed farms were also recorded.

Analyses of pesticide residues in soil samples. Analytical techniques. Analytical techniques used for the soil analysis at the laboratory involved extraction, identification, confirmation (wherever possible) and quantitation of pesticides. The studied pesticides included propamocarb, diazinon, pirimiphos-methyl, malathion, chlorpyrifos, phenthoate, triazophos, deltamethrin, cypermethrin, dimethoate and metalaxyl. Dimethoate and metalaxyl in the extract were separated and

Abstract. In the investigation of 40 different vegetable growing farms of Al-Batinah (Oman) and Al - Ain (UAE) regions different pesticide residues were found to be present on all soil samples which varied in their types and levels according to the region. In Omani soil samples, cypermethrin was the most frequent pesticide, followed by chlorpyrifos, malathion, phenthoate, triazophos and deltamethrin. In UAE farm soil samples, chlorpyrifos, cypermethrin and deltamethrin were detected in all the regions, while phenthoate was detected in the Eastern and Northern regions only.

Keywords: pesticide residues, cypermethrin, chlorpyrifos, deltamethrin, malathion, agricultural soil, Oman, UAE
Prevalence and Pathology of Helminth Infections in Pigs

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Abstract. In 30 viscera of local slaughtered pigs from different areas of Tangail and Mymensingh districts of Bangladesh, six species of helminths were identified; 2 of them were trematodes namely Fasciolopsis buski (36.70%) and Gastrodiscoides hominis (26.70%) and 4 species were nematodes namely Ascaris suum (60%), Metastrongylus elongatus (53.33%), Stephanurus dentatus (10%) and Physocyclus sexalatus (56.71%). Three nematode species, viz. M. elongatus extracted from lung, S. dentatus from peri-renal fat and P. sexalatus from stomach, were recorded for the first time in pigs in Bangladesh. No gross lesion was observed in pigs affected with M. elongatus. In A. suum infection, intestinal wall was infiltrated with plasma cells, lymphocytes and eosinophils. In M. elongatus infection, lymphocytes and macrophages mainly eosinophilic infiltration was observed in the parenchyma of lung. Age exerted a significant (p<0.05) influence on the development of the helminths, P. sexalatus, F. buski, A. suum and G. hominis.

Keywords: helminth infection, pigs, Bangladesh

Introduction

In Bangladesh, the pig rearing is limited to the minority people. Pig population in Bangladesh is estimated as 8 millions (Rahman, 2004). Some of the pig parasites have public health significance (Talbot, 1972). It is reported that most of the pig parasites are the causes of great economic loss in terms of poor growth and weight loss (Johnson et al., 1972). Internal parasites devitalize pigs by robbing them of essential nutrients and injuring vital organs. Pigs heavily parasitized are more susceptible to diseases such as scours and pneumonia (Soulsby, 1982). The resulting diseases and unthriftiness are a major cause of economic loss.

Bangladesh due to its conducive geo-physical condition and tropical climate is considered as haven of parasites (Salkeld et al., 2008). Situation is further aggravated by orthodox husbandry methods such as rearing pigs in clay, feeding the pig herd in dirty places etc. As such pigs remain susceptible to both ecto- and endoparasites. Basak (1988) has documented 5 species of parasites from visceral samples of pigs from some districts of Bangladesh. Moreover, Shaikh and Huq (1984) recorded Ascaris suum, Trichuris suis, Fasciolopsis buski and Ancylostoma duodenale. But unfortunately no attention has been paid to study the pathology of parasites in pigs in Bangladesh. The present study was aimed at determining the prevalence of helminth infections and pathological lesions produced by them.

Materials and Methods

For the study, a total of 30 viscera of slaughtered pigs were examined for helminths during the period from July, 2005 to May, 2006. Visceral examination, parasite identification and preservation were conducted in the laboratory at the Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, whereas, pathological study was performed in the Department of Pathology, Bangladesh Agricultural University, Mymensingh.

Post-mortem examination, parasite collection and identification. Viscera of 30 slaughtered pigs were collected from different places of Mymensingh and Tangail districts. In Mymensingh district the pigs are reared by lower cast Hindus and in Tangail district, non-Muslim ethnic people are engaged in the practice. These were the only two areas from where the visceral samples could be collected and brought to the laboratory within five to six hours and so it was possible to examine all the samples on the day of collection. Viscera were collected from local markets where the pigs are slaughtered. After collection the viscera were brought to the laboratory. Each part of the gastrointestinal tract was opened through long axis by giving longitudinal incision with scissors in separate clean buckets. The contents of the respective part were
Concentration of Electrolyte Reserves of the Juvenile African Catfish *Clarias gariepinus* (Burchell, 1822) Exposed to Sublethal Concentrations of Portland Cement Powder in Solution

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Abstract. The study investigated the effect of sublethal concentrations (39.10, 19.55, 9.87 and 0.00 mg/l) of Portland cement powder in solution on the electrolyte reserves (sodium, potassium, calcium, chloride and inorganic phosphorus) in the serum, liver and kidney of the juvenile African catfish *Clarias gariepinus* after a 15 day exposure period. The basic function of the determined electrolyte reserves in the body lies in controlling fluid distribution, intra and extra cellular acidobasic equilibrium, maintaining osmotic pressure of body fluid and normal neuro-muscular irritability. The result revealed significant (P<0.05) changes in serum sodium, potassium, calcium and chloride and insignificant (P>0.05) changes in inorganic phosphorus. Sodium, calcium, chloride and inorganic phosphorus and potassium were significantly (P<0.05) and insignificantly (P>0.05) different in liver and kidney, respectively. Ipso-facto, the effector organs viz: liver and kidney of teleost species – *Clarias gariepinus* which are primarily responsible for regulating water and ionic movement between external and internal milieu of fishes are susceptible to deleterious effects of Portland cement powder thus sublethal concentration (39.10 mg/l) of Portland cement powder in solution after a 15 day exposure has been most toxic and debilitating to the test fish.

Keywords: portland cement, electrolyte reserves, serum, liver, kidney, *Clarias gariepinus*.

Introduction

Though involved in the developmental structure of any country in the modern world but cement industry generates dust/aerosol during its operations. Portland cement also known as hydraulic cement is composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrate and gypsum with trace constituents like potassium oxide, sodium oxide, chromium compound and nickel compound (Mindess and Young, 1981). Use of these important ingredients of concrete and mortar as well, cannot be avoided in building construction. Cement and most of its constituents have been found to be toxic to living organisms in the environment (Adamu and Audu, 2008; Adamu and Kori-Siakpere, 2008; Akinola *et al.*, 2008; Khattak *et al.*, 2005, Fatima *et al.*, 2001; Hansen, 1998).

The development and severity of the injury caused by the cement dust on plants or animals depend besides the concentration of the pollutant, also on the duration of exposure to the pollutant, the species and its stage of development as well as the environmental factors conducive to the accumulation of the cement dust in the organism, which makes it susceptible or resistant to injury (Heather, 2003).

According to Musa and Omorogie (1999), fish are intimately associated with an aqueous environment and physical and chemical changes in the environment are rapidly reflected as measureable physiological changes in the fish.

Electrolyte reserves are the ions balance that change within certain limits depending on metabolic activities caused by some environmental factors such as pollution. In fresh water fishes, blood and electrolyte concentrations are regulated by interacting processes, such as absorption of electrolyte from the surrounding medium through active mechanism mainly at the gills and through selective re-absorption of electrolytes from urine. Any alteration in one or more of these processes results in a change in the plasma electrolyte composition. These ions play vital role in several body functions. The monovalent ions, sodium, potassium and chloride are involved in neuromuscular excitability, acid base balance and osmotic pressure (Verma *et al.*, 1981), whereas divalent ions such as calcium and magnesium facilitate neuromuscular excitability, enzymatic reaction and retention of membrane permeability. Inorganic phosphate acts as a major cytoplasmic buffer and is the basis of energy exchange (Aurbach *et al.*, 1985).

Despite the fact that cement production leads to the formation of aerosols which invariably reach the aquatic system, no detailed study has been reported on its sublethal effect on concentration of the electrolyte reserves of fish. Therefore, the present study seeks to determine the sublethal effect of Portland cement powder in solution on the concentration of...
A List of Damselflies (Zygoptera: Odonata) Recorded from Azad Jammu and Kashmir, Pakistan

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Introduction

Damselflies are predatory both in larval and adult stages. The larvae are voracious feeders and prey upon aquatic insects e.g., mosquito larvae and aquatic larvae of other species (Sahayaraj, 2004). Adults normally feed on small flying insects such as gnats (Meyer, 2005), mosquitoes and midges during flight (Pedigo, 2002). The presence of odonates is taken as an indicator of ecosystem health for both aquatic and terrestrial ecosystems (Watson et al., 1982). Adult damselflies feed on the insect pests of crops as well, especially of rice (Yousuf et al., 1998; Yasumatsu et al., 1975) and cotton (Yousuf et al., 1995; Yunus et al., 1980).

Odonates have been the focus of extensive research in many countries. They belong to one of the few insect orders that have been intensively studied in the tropics (Woodward, 2001). They have been documented on all continents except Antarctica and are usually concentrated in warmer, tropical habitats (Boyd, 2005). According to Sahayaraj (2004), approximately 5600 named species of Odonata have been described, so far, all over the world.

In the past, Fraser (1933-36) and Laidlaw (1915) reported Odonata from the subcontinent. Kanth (1985) studied the Odonata of Azad Jammu and Kashmir, but his survey was not comprehensive. Yousuf et al. (2000a; 2000b), Luqman (1995), Khalil et al. (1995; 1990), Khalil and Siddique (1995), listed odonates from different districts of Azad Jammu and Kashmir. However, this area has high topographic diversity and requires further surveys to reveal the existing zygopterous fauna of the region.

The distribution of damselflies is not well known in Azad Jammu and Kashmir. The valley is bestowed with diverse habitats and abundant streams and springs. The objective of the present study is to prepare a comprehensive and updated record of the damselflies (Zygoptera) of Azad Jammu and Kashmir.

Materials and Methods

Twenty-eight different sites (four sites per district), as follows, were selected to collect adult damselflies from the valley of Azad Jammu and Kashmir (Fig. 1), concentrating on the habitat requirements of damselflies.

District Poonch: Rawalakot (L1), Banjosa (L2), Hajira (L3), Abbasspur (L4).

District Sudhnoti: Tarar Khal (L5), Palanadri (L6), Goraha (L7), Azad Pattan (L8).

District Muzaffarabad: Chikar (L9), Chakothi (L10), Patika (L11), Muzafarabad (L12).

District Bagh: Arja (L13), Bagh (14), Bajri (15), Harighal (L16).

District Mirpur: Mangla (L17), Dudial (L18), Palak (L19), Azad Pur (L20).

District Kotli: Sensah (L21), Sarsawa (L22), Dongi (L23), Kotli city (L24).

District Bhimber: Samahni (L25), Barnala (L26), Kodala (L27), Bhimber city (L28).

Adult damselflies were collected during the summer season of three consecutive years, 2005-2007 (Table 1). Specimens were collected using aerial nets, sweep nets and dip nets when catching over water. The specimens were killed in cyanide bottles and transferred to paper bags for transportation to the laboratory. In the laboratory they were softened and rehydrated.

Abstract. In the intensive survey of the valley of Kashmir for updating the record of damselflies inhabiting the region, a total of 15 genera and 31 species of damselflies were collected during the summer season of three consecutive years (2005-2007) which are reported.

Keywords: damselflies, Zygoptera, Odonata, Azad Jammu and Kashmir, Pakistan

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Introduction

Alpha amylase catalyses hydrolysis of α-D-(1,4) glycosidic linkages in starch components and related carbohydrates. It is a key enzyme in the production of starch derivatives and also can be used in desizing fabrics, baking industry, pharmaceuticals and detergents. The growth and enzyme production of the organism are strongly influenced by medium composition thus optimization of media components and cultural parameters are the primary tasks in a biological process (Djekrif-Dakhmouche et al., 2006). The main strategy used is media engineering for which the operating conditions parameters are optimized by changing one parameter at a time and keeping the others at a constant level (Liu and Tzeng, 1998). Optimization studies do not take into consideration effects of interaction among different variables as any process can be influenced by several variables (Silva and Roberto, 2001). Limitations of single factor optimization can be eliminated by employing response surface methodology (RSM) which is used to explain the combined effects of all the factors in a fermentation process (Elibil, 2004). Single variable optimization methods are not only tedious, but also can lead to misinterpretation of results, especially because the effect of interaction between different factors are overlooked (Wenster-Botz, 2000). Response surface methodology may be summarized as a collection of experimental strategies, mathematical methods and statistical inference for constructing and exploring an approximate functional relationship between a response variable and a set of design variables.

Abstract. In this work, central composite design combining with response surface methodology was successfully employed to optimize medium composition for the production of alpha amylase by *Serratia marcescens* SB08 in submerged fermentation. The process parameters that influence the enzyme production were identified using Plackett-Burman design. Among the various factors screened, inoculum concentration, pH, NaCl and CaCl2 were found to be most significant. The optimum level of pH was 5.0, inoculum concentration 3%, NaCl 0.30 g/l and CaCl2 0.13 g/l. The actual enzyme yield before and after optimization was 56.43 U/ml and 87.23 U/ml, respectively. Thus, it is advisable to the microbial industry sponsors to apply such profitable bioprocess to maintain high yield for mass production of α amylase.

Keywords: alpha amylase; fermentation; *Serratia marcescens*; Plackett-Burman design; central composite design

Materials and Methods

Microorganism and culture maintenance. Potent strains of bacteria were isolated from the gut of sulphur butterfly (*Kricogonia lyside*). The sulphur butterfly was washed with 70% ethanol and with sterile distilled water several times to eliminate surface bacteria. All dissections were performed under sterile conditions. After disrupting the walls, the contents of the stomach were collected in sterile eppendorf tube, containing phosphate-buffered saline; contents were serially diluted, spread onto the surface of nutrient agar plates and incubated for 48 h at 30 °C in order to record total colony forming units (CFU/ml). The *Serratia marcescens* SB08 isolated from the gut of sulphur butterfly was maintained at 4°C on nutrient agar slants and subcultured every 2 to 4 weeks.

Amylase production. Five ml starch broth was inoculated with 1 ml of inoculum and was incubated at 30 °C for 18 h. This 5 ml
Short Communication

Beneficiation of Malakand Graphite Ore

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Abstract. On beneficition of an indigenous graphite ore of Malakand, Pakistan, on laboratory and pilot plant scale by froth flotation technique, the ore, which initially contained 17% graphitic carbon, was upgraded to a graphite concentrate containing 84% graphitic carbon with more than 70% recovery. The concentrate conforms to the specifications of the foundry grade graphite. Maximum recovery is achieved at pH 7.5 and pulp density of 33% solids with a dose of 0.14 kg/ton sodium silicate, 2.2 kg/ton kerosene oil and 0.02 kg/ton pine oil.

Keywords: froth flotation, graphite, beneficiation, Malakand graphite ore

Natural graphite is a form of carbon, found as crystals of high graphitic content or in amorphous form of lower purity. (Brady, 2004; Liu and Yuyan, 2003; Kalyoncu, 2001; Gordon, 1995). Due to its conductivity, high thermal stability and lubricating properties, graphite is used in various industries according to the grade. (Anwar et al., 2006; Kalyoncu, 2001; Gordon, 1995).

At present, Pakistan imports all its requirements of foundry grade graphite containing 75-85% graphitic carbon (Anwar et al., 2006; Kalyoncu, 2001; Crossley and Peter, 2000; Qureshi et al., 1967). In the Malakand area of North Western Frontier Province (NWFP) of Pakistan, an extensive deposit of low grade graphite ore, containing 10-17% graphitic carbon, has been found which can be exploited on commercial scale.

This paper deals with the beneficiation study of the said graphite deposit using froth flotation technique. Chemical analysis was carried out by conventional gravimetric and volumetric methods. Characteristics of the ore were defined by petrography, X-ray diffraction and ore microscopy. Flotation feeds of the ore were prepared by subjecting the ore to crushing and wet grinding at 1:1 solid/ liquid ratio. Flotation tests were carried out in a Denver D-12 flotation machine. The steps of beneficiation of the ore are given in the flowsheet. Optimum flotation parameters and metallurgical balance are presented in Table 1 and 2.

The grade (graphitic carbon=16.94%) of the ore (Table 3) is sufficient to exploit the ore on commercial scale (Hand, 1997; Hussain et al., 1967). The ore was found as fine grained graphitemica schist. The gangue mostly comprised of clay with hydrated oxides of iron and predominant quantities of silica and mica. The ore contains crystalline graphite

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Flowsheet: Beneficiation of Malakand graphite ore.
Sesquiterpenes.

Sesquiterpenes (STs) are 15 carbon terpenoids comprising of mainly two types, oxygenated sesquiterpenes and hydrocarbon sesquiterpenes. The oxygenated forms occur bearing functional groups such as alcohols, ketones, aldehydes, acids or lactones in nature. Due to their small molecular weight, they are important volatile organic parts of especially the essential oils, which are of great medicinal potential. Besides their presence in essential oils, they are also major constituents of various medicinal and economically important plants (Merfort, 2002).

STs are one of the largest biogenetically homogenous groups of natural products known. More than 11,000 entries of sesquiterpenes have been reported so far. These sesquiterpenes have been divided into almost 24 different kinds (Schmidt, 2006). However, germacranolides, eudesmane, farnesane, elemane, guaiane, and chamigrane are well known besides some esters or lactone group-linked sesquiterpenes. Almost 5000 sesquiterpene lactones have been reported. Among them 60 to 65% have been reported to be present as essential/edible oils (Schmidt, 2006; Fraga, 2000).

Various STs have been found to be biologically active against cell proliferation, abnormal cell growth (cancer), inflammation, bearing antibacterial, antifungal, antispasmodic, cytotoxic, antimalarial, hepatoprotective, insecticidal, allelopathic, enzyme inhibitory, antilipase effects as well as many other diseases and problems (Khan et al., 2008; Liu et al., 2008; Macias et al., 2007; Pan et al., 2007; Miguel et al., 2005; Ding et al., 2005; Rafi et al., 2005; Sharma et al., 2005; Yun et al., 2002a,b; Rafi et al., 2005; Sharma et al., 2005; Yun et al., 2002a,b; Rafi et al., 2005; Sharma et al., 2005; Yun et al., 2002a,b).

Antioxidants. Antioxidants are classically defined as molecules present in concentrations lower than the biomolecules and may prevent, protect or reduce the extension of oxidative damage, such as glutation peroxidase, catalase and superoxide-dismutase. Other antioxidants, such as ascorbic acid (vitamin C) and tocopherol (vitamin E) are non-enzymatic antioxidants (Hussain et al., 2008; Khan et al., 2007, 2006, 2005; Foyer and Noctor, 2005; Bolwell and Wojtaszek, 1997; Harborne, 1993). Thus, there is a delicate balance between the generation and destruction of oxidants, which may be beneficial or deleterious to the organism (Hussain et al., 2008; Khan et al., 2007, 2006, 2005; Maffei et al., 2007; Foyer and Noctor, 2005; Novelli, 2005). Oxidation products from lipids and cholesterol are thought to be the contributing factor to the cause of various diseases, including cancer, atherosclerosis and some age-related diseases (Chun et al., 2007; Jatoi et al., 2007; Pandhair and Sekhon, 2006; Ho et al., 2003; Andersson et al., 1996; Chan, 1987; Scott, 1985). Lipid oxidation in food affects its nutritional quality which results in rancid flavour, one of the main consequences. Also loss of vitamins, polyunsaturated fatty acids and other essential compounds can occur during the process (Khan et al., 2008; Manzoor et al., 2007; Pandhair and Sekhon, 2006; Anwar and Bhanger, 2003; Andersson et al., 1996; Chan, 1987; Eriksson, 1982). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalyses the reduction of molecular oxygen to super-
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