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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Distribution and Activity of Microbial Population for Jute Retting and Their Impact on Water of Jute Growing Areas of Bangladesh

M. Shamsul Haque, Zakaria Ahmed, Md. Asaduzzaman, M. A. Quashem and Firoza Akhter
Bangladesh Jute Research Institute, Manik Mia Avenue, Dhaka-1207, Bangladesh

Abstract: Microbial population varies from place to place in the jute growing areas of Bangladesh. Fungal load was higher in post retting water. The addition of post retting microbes in retting test, *in vitro*, accelerated the retting. Retting period was almost half in treatment with microbes from post retting water than that of pre-retting water. Chemical properties of post retting water was within the range of environmental control.

Key words: Jute retting, microbes, population, environment

Introduction

Microbes play an important role in retting of jute, while the plants are kept submerged in water the decomposition of the non-fibrous matters is brought about by the aquatic microbes predominantly the bacteria. Retting and fibre extraction problems are complex and multi-disciplinary in nature. Jute are retted microbially involving aerobic microbes initially but major task is performed by anaerobes. Retting does not produce any toxic substances and materials released during the process of retting are fully biodegradable. Jute wastes and by-products have manurial, cooking fuel, timber and pulp-wood substitute values, conserving forests and thus are environment friendly. However, there is transitory loss in retting water quality. During retting due to O₂ depletion the gill-breathing fishes may die but air breathing fishes can thrive. There are excessive microbial load and discolouration of water. Environmental aspect, therefore, should form important component of retting studies.

The impurities in water are discussed under three main headlines viz. a) turbidity and colour, b) iron and manganese and c) alkalinity and hardness. Retting in stagnant waters makes the water darker, there is bad odour and at times site unappealing. Due to organic mass released there is increased microbial load and prolonged depleted O₂ levels. After some time these negative effects disappear. Therefore the situation could be described as transitory polluting. However, there is inadequate data on the subject. A systematic study can therefore be helpful to respond to question on this aspect of retting which is likely to increase due to the growing environment consciousness. Thus the present study was undertaken to study the microbial population and their impact on water during jute retting in different jute growing areas of Bangladesh.

Materials and Methods

The experiment was carried out in the microbiology and biochemistry laboratory (Agriculture Wing), Central Research Station, Bangladesh Jute Research Station, Manik Mia Avenue, Dhaka-1207, Bangladesh for three consecutive years.

Collection and preservation of samples: Water was collected aseptically at pre-retting and post-retting condition from the same water bodies of different places of Jessore namely Rajganj, Keshabpur, Monirampur, Jhikargacha and from Alampur, Jugia, Shastipur and Vadalía in the district of Kushtia, Bangladesh. Ten ml of water from each sample was transferred to 250 ml conical flask containing nutrient broth for multiplication of microbes. After 7 days of incubation, 10 ml culture from each of the samples (both pre-retting and post-retting) were heated in water bath for 20 min at 80°C to limit the study to spore forming anaerobes only. Then plated out in nutrient agar by using diluted culture (1 in 10⁵). Representative colonies appearing after 7 days incubation, were counted to determine the population of anaerobes, unless stated otherwise. Cultures of anaerobes were incubated in specially made

anaerobic jar (BTL-anaerobic jar). For aerobic culture, Nutrient broth of all the samples were directly plated out without prior heating and incubated at 37°C in the presence of air in incubator. Representative colonies appearing after 7 days incubation were counted to determine the aerobic bacterial population (both pre-retting and post-retting cultures). Similarly fungal populations were also determined. For bacterial culture pH of the medium was 7.2 and for fungal culture it was 5.4. Water samples were collected from a depth of 10-15cm below the surface using a 250ml glass stoppered bottle with scale (Mishra *et al.*, 1992).

Preservation of samples is difficult because almost all preservatives interfere with some of the tests. Immediate analysis is ideal. Samples for dissolved oxygen (DO) determination were preserved as directed under oxygen (dissolved). Samples were preserved in chilled (3-4°C) condition immediately for biological oxygen demand (BOD) determination. Samples for chemical oxygen demand (COD) were preserved by adding sufficient H₂SO₄ to obtain a final pH of 2-3.

Determination of BOD, COD, DO and other gas: BOD were determined according to Mohlman *et al.* (1928) and DO were analyzed by Winkler or iodometric method which is a titrimetric procedure based on the oxidizing property of DO and estimated DO in µg L⁻¹ range (Winkler, 1888). On the other hand, the dichromate reflux method following APHA was used for the COD determination (Anonymous, 1989; Moore *et al.*, 1949). DO content of water was measured by DO meter (Model- JENWAY-9015). CO₃ and HCO₃ alkalinities and free CO₂ were determined by titrimetric method (Welch, 1948).

Determination of total and temporary hardness: Aqueous methyl orange indicator solution was added to 100 ml water sample and was titrated with standard dilute hydrochloric acid until the end point was found. A blank test was carried out using the same procedure. The blank correction was subtracted from the volume of standard acid used. The corresponding weight of calcium carbonate was then converted into parts per million (Uddin and Salam, 1989). Total hardness, calcium hardness and magnesium hardness were measured following Mishra *et al.* (1992).

Determination of retting efficacy: To determine the retting efficacy of the culture (both pre-retting and post-retting) pieces of 12 cm long jute stem (*Corchorus capsularis* var. CVL-1), weighing 40 g were used as retting materials. The stem pieces were placed into surface sterilized earthen plates (Sankee) containing 800 ml of distilled water. Ten ml of Nutrient broth culture of all samples were then added to each of the Sankee separately. Sankees were covered with another surface sterilized Sankee and kept at room temperature of 29-30°C. Progress of retting was tested aseptically 7 days after incubation. Fibre quality was assessed by "Touch and Feel" method (recommended by Bangladesh Jute Research Institute). In control, no bacterial inoculum was added, only 10 ml of distilled water was added. The test was replicated thrice.

Results and Discussion

Microbial population was higher in post retting water than in pre-retting water and in some places, particularly Alampur, Jugia and Vadalia, no anaerobic bacteria was found in pre-retting water (Table 1). Aerobic bacterial population was highest (2.9X10⁶) in pre-retting water collected from Jhikargacha of Jessore, while it was lowest in water samples collected from Shastipur of Kushtia

(2.5X10⁵). Post retting water collected from Monirampur of Jessore contained highest aerobic bacteria (6.0X10⁶) while that of Vadalia of Kushtia had lowest (1.6X10⁶) aerobic bacteria (Table 1). Anaerobic bacterial population was lowest (1.0X10⁵) in pre-retting water of Jhikargacha of Jessore and highest (5.9X10⁶) in post retting water from Manikganj of Dhaka. Fungal population was also highest in post retting water and no fungal population was

Table 1: Microbial population in different jute growing areas of Bangladesh.

Place	Aerobic bacteria		Anaerobic bacteria		Fungus	
	Pre-retting	Post retting	Pre-retting	Post retting	Pre-retting	Post retting
Jessore						
Jhikargacha	2.9X10 ⁶	4.2X10 ⁶	1.0X10 ⁵	4.2X10 ⁶	Nil	1.5X10 ⁵
Keshabpur	2.1X10 ⁶	3.0X10 ⁶	5.5X10 ⁵	1.7X10 ⁶	1.0X10 ⁵	3.0X10 ⁵
Monirampur	2.7X10 ⁶	6.0X10 ⁶	2.0X10 ⁵	3.1X10 ⁶	2.0X10 ⁵	3.0X10 ⁵
Rajganj	1.7X10 ⁶	3.2X10 ⁶	5.5X10 ⁵	1.0X10 ⁶	1.0X10 ⁵	3.0X10 ⁵
Kushtia						
Alampur	1.1X10 ⁶	2.4X10 ⁶	Nil	4.0X10 ⁵	2.0X10 ⁵	3.5X10 ⁵
Jugia	4.0X10 ⁵	3.8X10 ⁶	Nil	6.0X10 ⁵	Nil	2.0X10 ⁵
Vadalia	3.0X10 ⁵	1.6X10 ⁶	Nil	1.3X10 ⁶	1.0X10 ⁵	4.0X10 ⁵
Shastipur	2.5X10 ⁵	2.6X10 ⁶	3.0X10 ⁵	2.4X10 ⁶	1.5X10 ⁵	4.5X10 ⁵
Dhaka						
Gazaria	1.5X10 ⁵	2.0X10 ⁶	2.9X10 ⁵	4.3X10 ⁶	3.7X10 ⁵	3.2X10 ⁶
Daulatkandi	1.2X10 ⁶	2.9X10 ⁶	5.3X10 ⁴	2.5X10 ⁵	2.2X10 ⁵	6.6X10 ⁶
Arikhola	1.6X10 ⁵	2.3X10 ⁶	3.2X10 ⁵	3.3X10 ⁶	4.1X10 ⁶	5.8X10 ⁶
Kaoraid	1.9X10 ⁶	5.0X10 ⁶	4.1X10 ⁵	4.6X10 ⁵	3.0X10 ⁵	3.3X10 ⁶
Sreepur	2.3X10 ⁶	2.4X10 ⁶	2.2X10 ⁵	3.9X10 ⁶	2.8X10 ⁵	4.5X10 ⁶
Manikganj	2.4X10 ⁵	3.7X10 ⁶	3.3X10 ⁵	5.9X10 ⁶	2.3X10 ⁵	3.6X10 ⁶

Table 2: Impact of microbial population on jute retting and production of quality fibre*.

Place	Pre-retting water		Post-retting water	
	Retting period (Days)	Fibre quality**	Retting period (Days)	Fibre quality**
Jessore				
Jikergacha	27	C*	10	B
Keshabpur	28	C	8	A
Monirampur	27	C*	8	B*
Rajganj	29	C	9	B
Kushtia				
Alampur	28	B	11	A
Jugia	27	C	8	B*
Vadalia	29	B	9	B
Shastipur	27	C*	8	B*
Dhaka				
Gazaria	28	C*	8	B
Daulatkandi	27	C*	9	B*
Arikhola	30	C*	10	B*
Kaoraid	29	B	11	B
Sreepur	30	C	9	A
Manikganj	29	C*	8	B*

* Average of three replications. ** A* = Best grade fibre. A= Best grade fibre but inferior to A* grade.
 B* = Good grade fibre but inferior to A grade. B= Good grade fibre but inferior to B* grade.
 C* = Poor grade fibre, inferior to B grade. C= Bad grade fibre.

Table 3: Chemical properties of post-retting water from different areas of Bangladesh*.

Place	Dry residue (g)	Temporary hardness (ppm)	Permanent hardness (ppm)	O ₂ (ppm)	CO ₂ (ppm)	Bicarbonate (ppm)	N ₂ (%)	Ca (ppm)	Fe (%)	BOD (mg/L)	COD (mg/L)
Jessore											
Jikergacha	10	7.20	70.00	3.00	14.00	105.00	0.0286	13.50	0.20	5.1	940.00
Keshabpur	9	7.50	78.00	1.00	17.00	102.00	0.0321	28.50	0.15	5.7	980.00
Monirampur	10	8.10	85.00	1.00	16.00	108.00	0.0357	15.00	0.18	6.6	898.00
Rajganj	10	7.30	76.00	2.00	15.00	103.00	0.0271	21.30	0.19	7.1	873.00
Kushtia											
Alampur	8	8.00	80.00	2.00	11.00	106.00	0.0331	17.60	0.22	5.4	971.00
Jugia	9	7.00	77.00	1.00	18.00	100.00	0.0275	19.40	0.16	6.7	966.12
Vadalia	11	7.20	83.00	3.00	15.00	110.00	0.0319	18.10	0.13	7.4	888.70
Shastipur	10	8.20	73.00	1.00	10.00	105.00	0.0224	19.70	0.21	7.2	979.32
Dhaka											
Gazaria	8	6.00	18.50	2.80	4.40	36.60	0.0142	15.70	0.18	8.1	889.43
Daulatkandi	10	4.70	45.00	3.00	8.60	134.20	0.0145	21.50	0.21	5.7	997.12
Arikhola	11	8.50	53.00	2.20	17.60	91.50	0.0214	16.30	0.19	6.5	986.04
Kaoraid	9	6.70	50.00	3.80	10.30	112.92	0.0148	18.40	0.15	6.8	971.34
Sreepur	10	5.40	91.00	4.20	12.20	110.50	0.0285	15.00	0.22	7.2	967.81
Manikganj	9	4.60	40.00	3.80	15.40	121.40	0.0107	19.00	0.14	5.6	891.10

* Average of three replications, BOD = Biological oxygen demand, COD = Chemical oxygen demand

obtained in pre-retting water collected from Jhikargacha of Jessore and Jugia of Kushtia. Fungal population was highest in pre-retting water from Arikhola (4.1×10^9) of Dhaka and highest (6.6×10^9) in post retting water collected from Daulatkandi of Dhaka. In the retting tests, it was observed that nutrient broth cultures of pre-retting water took longer time (27 to 30 days) in retting while in post-retting cultures retting was completed in 8 to 11 days. Fibre quality was also better in retting with post retting culture (Table 2). The presence of active microbial population showed great promise of an active inoculum to accelerate retting and improve the fibre quality. These microbes could be absorbed in substrate like wheat or rice bran and can be dehydrated using freeze dryer and if these are viable for long time, then these could be used in retting in stagnant water.

Hardness in water arises from the presence of calcium and magnesium salts. Calcium and magnesium carbonate are virtually insoluble in water. Hardness caused by bicarbonates is called temporary because it disappears on boiling. On the other hand, hardness of water due to calcium and magnesium chloride salts is called permanent hardness. Most water contain both temporary and permanent hardness (Goetz and Smith, 1959). Hardness may range from zero to hundreds of milligrams of calcium carbonate per liter depending on the source and treatment to which the water has been subjected (Snell and Biffen, 1944). Dissolved oxygen (DO) levels in natural and waste waters are dependent on the physical, chemical and biochemical activities prevailing in the water. The analysis for DO is a key test in water pollution control activities and waste water treatment process control (Mancy and Jaffe, 1966). The specific impurities of water which affect the quality, texture and uniformity of fibre include a) turbidity, b) colors, c) dissolved solids content and d) suspended materials. From Table 3, it was observed that the BOD, COD and all other chemical properties of post retting water were within the reach of environmental control (Fuller, 1974). Since, the oxygen dissolved in water is used for respiration by aquatic plants and animals, any added material that uses oxygen will interfere with the normal and natural growth of the aquatic organisms. Fish require the largest amounts of DO, invertebrate animals (protozoa, worms, insects, shrimps, etc.) can live with less, and bacteria can function with least of all. For a diversified population, including game fish, the amount of DO in water should be at least 5.0 ppm that is, 5.0 g oxygen for each 1.0 L water. If the DO present in a sample of water is lower than this amount, fish suffer most and tend to die out. The populations of invertebrates and bacteria then rise to abnormal levels (Fuller, 1974). The amount of DO used up during the oxidation by bacterial action of the organic matter present in a sample of water is called the BOD and water is considered pure if its BOD is 1.0 ppm or less, fairly pure with a BOD of 3.0 ppm, and of doubtful purity when the BOD is as much as 5.0 ppm. Discharge of waste water with a BOD of 20.0 into a stream is considered undesirable by public health authorities (Fuller, 1974). When larger than normal concentrations of nitrogen and phosphorus are present in water, several species of algae grow very rapidly and these produce a bloom. When the bloom dies and decays, it makes heavy demands on DO. The consequent lowering of the oxygen content of the water upsets the desirable balance of aquatic organisms.

Bhouyain (1983), Begum and Hossain (1993) worked on different industrial pollution of Bangladesh and they noted that industrial effluent can easily change water quality, increase anoxic condition of water and load of biodegradable and non-biodegradable organic and inorganic matter. The BOD and COD value indicate a greater degree of pollution by biodegradable and non-biodegradable organic matter (Chowdhury and Zaman, 2001). Retting in stagnant waters makes the water darker, there

is a bad odour, and in times the retting site unappealing. Due to organic mass released there is increased microbial load and prolonged depleted O_2 levels. After some time these negative effects disappear. Therefore the situation could be described as transitory polluting. When stems of jute are put in water for retting, there are two changes in the water quality. The first stage is that organism in green plants are dissolved and they produce plenty of nutrients for the growth of microbes. This promotes exuberant microbe multiplication, which consumes all of the DO in water; secondly, since organism in green plants degrade, the content of BOD, COD, sulphide, NO_3-N , total remains and suspend substances in the water greatly increases, and this seriously polluted the water. Since organisms in green plants are decomposed by microbes, the harmful substances in water, such as NO_3-N , sulphide, etc. are greatly increased with degradable action of microbes, but it required 30 days for other harmful substances to begin to decrease, except sulphide which begins to decrease after 8 days. After 131 days, they still are at a high level, and they cannot meet the hygienic standard of drinking water until after 217 days (IJO, 1994). After retting the stems or ribbons are washed. At this time a great amount of biological materials, such as wood and small pieces of plant are released into water. This cause a second pollution to the water quality.

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