Development of a Technology for Coconut Coir Retting using Consortium of Microorganisms

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ABSTRACT

A study was conducted to identify a microbial consortium that can enhance the coir retting process. In this, pilot scale retting trials were performed for coconut husks of Sri Lankan tall cultivar (CRIC 60) utilizing twelve microbes isolated from natural retting pits located in different parts of the country. Effectiveness of these microorganisms in coir retting was evaluated by inoculating them individually and in combinations of two/three strains and retting for a one month period. Innoculated samples were collected at biweekly intervals to evaluate the fibre extraction using the traditional Ceylon drum. The fibre samples were observed visually and microscopically for the pith content adhered to the balace and the fiber and they were also analyzed for percentages of bristle fiber, balace , pith content and pectin content. The ret liquor samples were also analyzed to determine pH, EC, total plate count and tannin content. Results show that the good quality bristle fiber can be obtained using consortium of microorganisms namely, *Bacillus firmus*, *Bacillus badius* and *Bacillus macerans* within a period of from 21 days.

Key words: *Coconut; retting; microorganisms; polyphenolic compound, Bacillus species.*

INTRODUCTION

Coconut (*Cocos nucifera* L) husk is a unique raw material for extraction of coir fiber. Coir fibre is extracted from monocarp of 1"-1¾" thick coconut husk. It is lignocellulosic and has a great demand world over due to its quality characteristics such as strength, resiliency, heat and dampness resistance and microbiological degradation (Meenatchisunderam, 1979). Basically, coir fiber is broadly classified into two different categories, bristles and mattress fibre. Sri Lanka is well-known in the world market for its high quality bristle fiber. The bristle fiber enjoys a good demand as it is the raw material for a range of high value end products. Extraction of bristle fibre is a little tedious as it requires soaking of the husk in a 'retting pit' for several months. Normally it takes more than six months to complete retting which enables the pith to be detached from the bristle fibre.

Retting is primarily a process leading to the degradation of pectin and polyphenolic compounds associated with the coir fibre. During coir retting it undergoes two distinguished physical and biological changes. In the physical change the husks absorb water and become swollen. Water soluble substances, carbohydrates tannins, nitrogenous compounds etc. leaches out of the tissue at this stage. During the second stage, biological changes occur with a variety of microorganisms developing in leached out substances. These micro-organisms act upon the substances that bind the fibers (Meenatchisunderam, 1979).

In Sri Lanka most of the retting pits occur as natural pits in lakes of fresh, brackish and saline water. Since the retting process takes a long duration, there is a demand from the industry to reduce this to a short period of time. This could be possible through making changes to the microbial action in the retting process. In actual fact, many different organisms could occur in the retting pits that are more efficient than the others in decomposing husks. Therefore, it is worthwhile to conduct a screening study to identify them and evaluate their activity. From the previous studies of Farook (2003), twelve microbial strains that may be capable of degrading pectin and phenolic compounds were identified. There is a need to assess their effectiveness in the retting process to select them for mass culturing. The objective of this study was to selectively identify the most active microbial strains to reduce the retting process time to a few weeks using individual and combinations of microbes.

MATERIALS

Coconut husks of Sri Lankan tall cultivar (CRIC 60) were used for this study. Twelve microorganisms identified from the previous studies of Farook (2003) were selected for the experiments as given in Table 1. Nutrient Broth, Plate Count Agar were used as the media for microbiological analyses.

Table 1. *Microbial strains selected for retting huskes in Experiment 1*

Bacterial species	Microbial Code (M. code)
Bacillus polymixa	1
Bacillus firmus	2
Bacillus subtilis	3
Kurthia sp.	4
Serratia liquifaciens	5
Bacillus badius	6
Bacillus sphaericus	7
Bacillus macerans	8
Escherichia coli	9
Enterobacter spp.	10
Micrococcus varians	11
Pseudomonas aeriginosa	12
Mixed culture of above 12 species.	13
Control (water with nutrient broth)	14

EXPERIMENTS

Experiment 1: Coconut husks retting by inoculating microbial strains.

Altogether there were 14 treatments, 12 inoculated with individual microbes, one with a mixed and uninoculated control. In each case, 15L of fresh water was inoculated with 30 ml of each culture medium. For each treatment five coconut husks were soaked in a bucket and kept for a period of one month. Total Plate Counts of the ret liquor samples were taken at weekly intervals. After 14 days of retting, two coconut husks from each treatment bucket were defibred using a Ceylon drum system. The defibred samples were subjected to visual observation of the balace to see the effectiveness of retting. The rest of coconut husks in each treatment were allowed to undergo retting up to 28 days. The retted husks were defibred and the extracted fibre and balace samples were subjected to visual observation. Percent (%) of bristle fibre, mattress fibre and pith content were measured by hackling 10g of husk samples which were defibred by using only the "breaker drum" of the traditional Ceylon drum system consisting of two drums namely "breaker" and "cleaner drums"

Experiment 2: Coconut husks retting by inoculating in combination of two microbial strains

In the second experiment 11 different treatments were made out of combinations as given in Table 2. Ten Combinations were made by mixing most effective microbial strains selected from the results of the first experiment. The 11th was the mixed culture of all the strains. After 21 days of retting 4 husks were subjected to defiber and the pith content adhered to the balace was visually observed. The rest was beaten by a wooden mallet for the determination of removable pith content.

Analysis was made to find the pectin content of the fiber as describe by Ranganna, 2000. Visual observation of the pith content adhered to the fiber was made with a microscope.

Table 2. *Combination of Microbial Strains for retting coconut husks in Experiment 2*

Microbial Combination	Sample Code
Pseudomonas aeriginosa & Serratia liquifaciens	A
Pseudomonas aeriginosa & Bacillus badius	B
Pseudomonas aeriginosa & Bacillus firmus	C
Pseudomonas aeriginosa & Bacillus macerans	D
Serratia liquifaciens & Bacillus badius	E
Serratia liquifaciens & Bacillus firmus	F
Serratia liquifaciens & Bacillus macerans	G
Bacillus badius & Bacillus firmus	H
Bacillus badius & Bacillus macerans	I
Bacillus firmus & Bacillus macerans	J
Mixed culture of 12 species (given in table 1)	K.

Experiment 3: Coconut husks retting by inoculating most effective microbial strains selected from the Experiment 2.

In this experiment there were 4 treatments of which three were made out of combinations as shown in Table 3. The last was kept as the control. Combinations were made from the most effective microbial strains selected from the results of the Experiment 2.

Samples were defibred after 21 days of retting. Fibre and the balace samples were visually observed for the pith content adhered to each sample. pH, EC, tannins (AOAC, 1999) and Plate Count were tested for the ret liquor samples at weekly intervals.

Coir fiber (treated from microbial strains in sample code R) was tested for the tensile strength and physical properties such as colour, length and impurities. Tensile strength was tested using the "Tensile Strength Tester" (Model: SM/100). Colour, length & impurities were tested according to the SLS 115: Part 1:1981. The parameters of coir fiber (treated from microbial strains in sample code R) were tested as the treatment gave good quality coir fiber.

Table 3. *Microbial consortia used for retting coconut husks in Experiment 3.*

Microbial combinations used	Sample code
Bacillus firmus (2) ,*Bacillus macerans* (8), *Bacillus badius* (6) & *Serratia liquifaciens* (5)	P
Bacillus firmus (2) ,*Bacillus macerans* (8) & *Serratia liquifaciens* (5)	Q
Bacillus firmus (2) ,*Bacillus macerans* (8) & *Bacillus badius* (6)	R
Control (With out microorganisms)	S

RESULTS AND DISCUSSION

Results of Experiment 1

The results obtained from experiment 1 is presented in Tables 4 - 7.

The categorization as in Table 4 was taken according to the ascending order of the pith content adhered to the balace samples. Microorganisms capable of degrading and decomposing pectin and phenolic compounds lead to the removal of the pith content adhered to the fiber of coconut husks. Pith content is one of the most effective parameter in the evaluation of the effective microbial consortia for the retting process. According to Table 4 treatment no.4 and 12 appear most effective in retting due to the least amount of pith content adhered to the balace. Hence, microbial strains

Kurthia sp (Microbial Code 4) and *Pseudomonas aeriginosa* (Microbial Code 12) were the most effective. But it was noted that the husk samples were not retted enough to give the good quality coir fiber. Thus the period of retting time was extended from 14 days to 28 days.

Table 4: *Ranking order of the effectiveness of microbial strains for reducing the retting time by visual observation of the balace after14 days of retting*

Treatment No.	
4 / 12	Most acceptable
2 / 13	↓
6 / 14	↓
3 / 11	↓
5 / 8	↓
1 / 10	Less acceptable

According to Table.5, Treatment no. 2 and 8 had higher amount of bristle fiber content than the other treatments. Hence *Bacillus firmus* (M.code no.2) and *Bacillus macerans* (M.code no.8.) were the most effective microbial strains for retting of husks.

Table 5. *Percent distribution of bristle, balace and the pith of the treated samples after 28 days of retting*

Treatment no.	Bristle fiber %	Balace %	Pith %
01	37.7	30.2	32.0
02	45.5	25.2	29.3
03	35.7	21.4	42.9
04	29.8	24.1	46.1
05	39.7	29.4	30.9
06	37.0	20.6	42.4
07	39.7	27.0	33.2
08	43.4	21.5	35.1
09	41.6	28.8	29.5
10	30.5	27.0	42.5
11	34.0	21.9	44.1
12	38.3	30.9	30.7
13	39.5	27.4	33.0
14	42.4	26.5	31.1

The above categorization was taken according to the ascending order of the pith content adhered to each balace samples (Table 6). According to the results there were four or five treatments that can be combined into one group. This is due to the insignificant variations of pith content in each balace sample. The balace samples grouped as X 1 had the least amount of pith content while Z 1 had the highest.

Table 6. *Categorization of the effectiveness of the sub grouped microbial strains for reducing the retting time by visual observation of the balace after 28 days of retting.*

Group No.	Treatment no.	
X 1	8 , 5, 9,13, 6	Acceptable
Y 1	4, 2,11,12	↓
Z 1	3, 1,14,7,10	Less acceptable

According to the results treatments 8, 14, 5, 12, 6, 13, 2, 1and10 can be combined into a single group (X 2) as there were insignificant difference in the pith content adhered in each fiber samples (Table 7). Based on that, microbial strains 7, 9 and 11 were put into one group (Y2) and it was noted that the pith content adhered to the fiber was higher than that of in X2 group. Samples treated from microbial strains 3 and 4 (Z 2) had highest amount of the pith content.

Table 7. *Ranking of treatments by visual observation of the fiber after 28 days of retting*

Group No.	Treatment no.	
X 2	8, 14, 5,12, 6,13 2,	Acceptable
Y 2	1,10	↓
Z 2	7, 9,11	Less acceptable
	3, 4	

The most effective microbial strains selected from the results of the 1st experiment were *Bacillus macerans* (8), *Bacillus badius* (6), *Bacillus firmus* (2) *Serratia liquifaciens* and *Pseudomonas aeriginosa*.

However, from this experiment alone it was not able to get high quality bristle fiber. So the investigation of retting the coconut husks was extended by using different combinations to find the effectiveness of these microbial strains.

Results of Experiment 2

Results of the experiment 2, are presented in Tables 8 to 10

Table 8 shows the categorization of the balace treated from the combinations of two microbial strains. In this experiment it was observed that there was least amount of pith content in the balace treated with G and E combinations while the highest amount of pith content was observed with A and K combinations. Thus microbial strains inoculated in sample G and E were most effective in enhancing the retting process.

Table 8. *Ranking order of the effectiveness of microbial combinations for reducing the retting time by visual observation of the balace after 21 days of retting*

Sample Code	
G , E	Most acceptable
F, J , C	↓
H , I	↓
A , K	↓
B	Least acceptable
D	

It was observed that the combinations J, C and G (Table 9) had higher amount of removed pith than others. Higher amount of removed pith content indicates more effective retting due to the microbial strains.

Table 9. *Percentages of removed pith content in fiber sample*

Sample code	Removed pith content from 10 g of husk
A	58%
B	66%
C	88%
D	63%
E	54%
F	21%
G	81%
H	57%
I	53%
J	89%
K	32%

Degradation of pectins occur due to the effect of the retting process. Therefore, pectin content of the fiber was analysed to find the effectiveness of microbial strains in the retting process.

According to degradation of higher amount of pectin (Table 10). the effectiveness of retting in samples decreases as follows:

A > E > F > D > J > C > B > K > H > G > I

Table 10. *Pectin content (initial pectin content= 1.4632g) in samples treated with different combinations of microbes.*

Sample Code	Pectin remained in 10 g of fiber sample	Pectin removed in 10 g of fiber sample	Percentage of Pectin Removed
A	0.0180	1.4452	14.452
B	0.4128	1.0504	10.504
C	0.3158	1.1474	11.474
D	0.1962	1.2670	12.670
E	0.0876	1.3756	13.756
F	0.1160	1.3472	13.472
G	0.4650	0.9982	9.982
H	0.4616	1.0016	10.016
I	0.6954	0.7678	7.678
J	0.2200	1.2432	12.432
K	0.4280	1.0352	10.352

The effectiveness of the samples from the microscopical observation are as followes.

J > G > C > B > D > H > A > E > I > K > F

According to these results it was determined that the microbial Strains *Bacillus macerans, Bacillus badius, Bacillus firmus and Serratia liquifaciens* are very effective in retting process

Results of Experiment 3

A third experiment was conducted to obtain the best quality fiber, as there was no significant variation in the quality of the fiber relative to fiber extracted after 28 days of retting, as given in the first experiment.

Mechanical extraction of the fiber after 21 days of retting

In the third experiment (Table 11) the visual observations of the pith content adhered to the balace and the fiber samples were made.

The husk Sample inoculated with microbial strains containing *Bacillus firmus* (2) ,*Bacillus macerans* (8) and *Bacillus badius* (6) had the least amount of pith content. Thus the microbial strains in this combination were very effective in enhancing the retting process. It was also noted that the quality of the fiber satisfy the requirements for the bristle fiber (Table 12).

An analysis of the ret liquor was conducted, as the condition of the ret liquor is changed. So this caused us to test the physico chemical parameters of the ret liquor sample. Total Plate Count (Fig. 1), pH variation (Fig. 2), Electrical Conductivity (Fig. 3), Tannins (Fig. 4) were tested to find their variation with time.

Table 11. *Pith content adhered to the balace and the bristle fiber from visual observation in samples treated with different combinations of microbes.*

Sample Code	Pith content
Sample treated from Bacillus firmus (2), Bacillus macerans(8) & Bacillus badius (6) - (R)	Less
Sample treated from Bacillus firmus (2), Bacillus macerans(8) & Serratia liquifaciens(5) – (Q)	↓
Sample treated from Bacillus firmus (2), Bacillus macerans(8), Bacillus badius (6) & Serratia liquifaciens (5)- (P)	
Control (With out microorganisms)-(S)	High

Fig.1 showed that Total Plate Count in ret liquor samples inoculated from the combinations of (R) and (Q) had increased with time. This is due to the growth of microbial strains by decomposing pectin & phenolic compounds. These poly pheonolic compounds cause to increase the pH value of ret liquor samples. The pH value of the ret liquor sample inoculated from the different combinations of the microbial strains is given in Fig.2. Tannins is one of the such polypheonolic compound that had affected an increase in the pH value of ret liquor.

Table 12. *Psychical and strength properties of the various types of bristle fiber in the sample treated with Bacillus firmus,Bacillus macerans* and *Bacillus badius*

Parameter	Test sample	Industrially used coir fiber	SLS Standards for Bristle Fiber
Tensile Strength	34 N	41 N	NNA
Colour	Reddish brown	Reddish brown	Reddish brown
Impurities	5.9%	5.7%	5.5.5%
Length	Long - 18%, Medium - 61%, Short-21%	Long - 20%, Medium - 59%, Short-18%	Long - 30%, Medium - 15%, Short - 44%

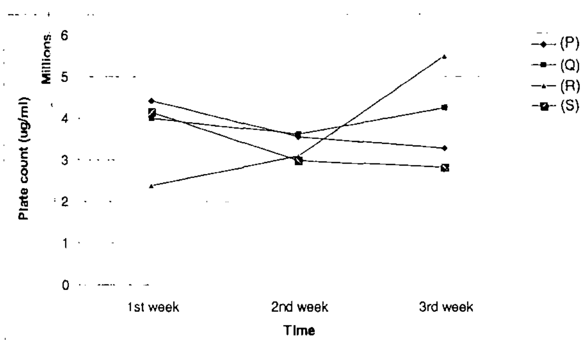

Fig.1. *Variation of the Total Plate Count in ret liquor samples inoculated from different microbial strains*

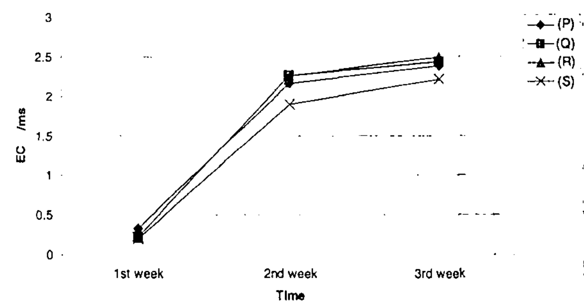

Fig.3. *Variation of the Electrical Conductivity with time in ret liquor samples inoculated from different combinations of microbial strains.*

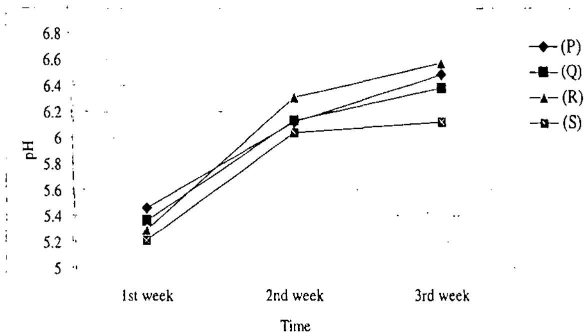

Fig.2. *Variation of the pH in ret liquor samples inoculated from different combinations of microbial strains.*

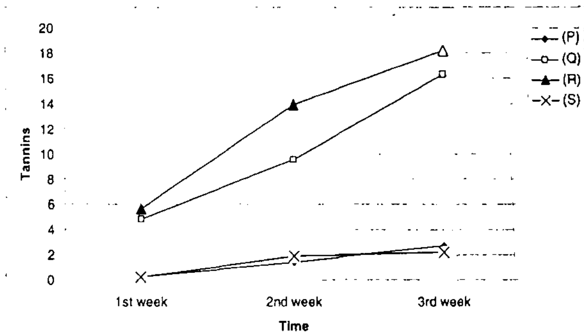

Fig.4. *Variation of the tannins with time in ret liquor samples inoculated from different combinations of microbial strains.*

Electrical conductivity of ret liquor samples increases with time due to ions such as Ca^{++}, Mg^{++} and K^{+}. Results showed that the electrical conductivity of the ret liquor samples inoculated from the combination "R" has the highest value (2.49m/s) (Fig.3).

Results (Fig.4.) show that tannins in all samples were increased with time. It is because of the addition of polyphenolic compounds by the degrading coconut husks. From the results of pith content adhered to the fiber & the balace, Plate Count, EC, pH and Tannins, the most effective microbial consortia were selected in the descending order (Table 13)

Table 13: *Most Effective consortia of Retting Enhancing Microbes*

Group No.	Consortium of microbes	
1.	Bacillus firmus(2), Bacillus macerans (8) and Bacillus badius (6)	More acceptable
2.	Bacillus firmus(2), Bacillus macerans (8) and Serratia liquifaciens(5)	↓
3.	Bacillus firmus(2), Bacillus macerans (8), Bacillus badius (6) and Serratia liquifaciens(5)	↓
4.	Control	Less acceptable

CONCLUSIONS

The consortium of microorganisms comprising *Bacillus firmus*, *Bacillus maceran* and *Bacillus badius* significantly enhances degrading and decomposing pectin and phenolic compounds in retting of coconut husks.These selected microbial strains survive in the pH range 5-7. The shortning of the duration of retting process in natural pits from more than five months to 21 days is a significant break through in the coconut coir processing technology.

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