DESIGN OF SUSTAINABLE INDUSTRIAL SCALE BIOCATALYSIS BASED FLAX RETTING PROCESS

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ABSTRACT: The aim of this applied research is to design a sustainable industrial scale enzyme based flax retting process. A systematic approach has been adopted. The screening and selection of enzymes for flax retting has been carried out. Alkaline pectinase has been identified as the most appropriate enzyme for the flax retting purpose. Optimisation of process parameters has been carried out using alkaline pectinase, non-ionic surfactants and chelating agents in terms of concentration of enzyme and other auxiliaries, time, temperature, liquid to solid ratio etc. Scale up experiments were performed. The BOD, COD and $N_{Kjeldahl}$ of the process waste water have been evaluated. At the end, an eeconomical evaluation of the successful flax retting process has been performed.

Key Words: Flax fibres, Retting, Biocatalysis, Alkaline pectinase, Scale up, Economic feasibility study

1. INTRODUCTION

The majority of the flax fibres used in the linen industry worldwide is grown in the Netherlands, Belgium and Northern France. An important step is the retting process wherein said flax fibres are prepared for further processing. At present there are two different ways of flax retting: dew retting and water retting. For dew retting the stems are laid on the ground up to six week for exposure to sun and dew. In water retting the straw are tied into fairly large bundles and steeped in water for up to 10 days. The water retting process is highly labour intensive and leads to extreme water pollution. For dew retting, which is the most common process, the expected retting time is very high from 10 days until 6 weeks with the chance of poor quality and lower yield of retted flax fibres as a result of bad weather conditions. The goal of this study was to design a new industrial process based on enzyme technology. The overall research and development work were divided into following stages. A) Selection and screening of enzymes for retting flax. B) Optimization of the reaction parameters for the selected enzymes, such as: pH, enzyme concentration, time, process temperature, liquid-solid ratio for the process, addition of surfactants and chelating agents etc. C) Scaling up experiments. D) Designing an industrial plant and performing an economic feasibility study.

2. MATERIALS AND METHOD

2.1 Materials

Fully dried and mature flax stems were supplied by Filo engineering, Arnhem. The flax stems were stored in dry location without being in contact with water to avoid any microbial influence. Based on a literature survey [1], it was found that alkaline pectinase (pectate lyase) is the most suitable enzyme for degradation of the pectine backbone of flax fibre hence suitable for flax retting. Hence, commercially available pectate lyase (EC 4.2.2.2) was

procured from Novozyme, Denmark. The commercial name of the enzyme is Scourzyme-L. Scourzyme-L has 275U/ml activities and it is most effective at pH 8, temperature around 40°C. The following chemicals and auxiliaries were used during enzymatic retting process. Tris(hydroxymethyle)aminomethane (99%+ TRIS) was purchased from Acros organics. TRIS was used for making an alkaline buffer solution. HCl/ Hydrochloric acid (25% solution in water) was purchased from Acros Organics. EDTA (Ethylene Diamine Tetra-acetic acid) is a Ca^{++} ionchelator and was purchased from Thermo-Scientific, the Netherlands. Triton® X-100, a non-ionic surfactant, was acquired from Acros organics, the Netherlands.

2.2. Methods

2.2.1: Buffer preparation: All the retting experiments were performed in alkaline 0.5M Tris-HCl buffer solution at pH 8.0 and pH 8.2. To prepare 2 litre of 0.5M Tris-HCl buffer, 12.14g of TRIS was dissolved in 1 litre demi water, the pH was monitored by a digital pH meter (Hanna Instruments, pH 209, pH meter). The desired pH of 8.0 or 8.2 was adjusted by adding diluted HCl in demi water. At the end, the final volume of 2 litres was made by adding the remaining demi-water. The buffer was stored in a cool dry place and was consumed within 7 days from the preparation. The freshly prepared buffer was used as a bulk medium for the flax retting, by adding several chemicals, auxiliaries and/or enzyme.

2.2.2 General enzymatic retting procedure: The flax retting process was carried out in a glass beaker (Figure 1). 2g of chopped flax stems were collected from the flax bundle. All the flax retting experiments were carried out in 0.5M Tris-HCl buffer medium at pH 8.0 or 8.2. The solid to liquid ratio was varied from series to series from 1:5, 1:10, 1:15, 1:20, to 1:40. The flax retting experiments were conducted at varied temperature in a water bath at 20°C, 30°C, 50°C and 60°C. The time for flax retting was varied from 30min, 1hr, 2hr, 4hr, 6hr, 9hr, 15hr, 24hr, 48hr, and 72hrs and up to 96hrs (4 days). The pectinase (Scourzyme L) concentration was varied from 0.5ml, 1ml, 1.5ml, 2.0ml and 2.5ml per experiment for 2 grams of flax. Flax retting experiments were conducted in combination of enzyme alone, or enzyme and non-ionic surfactant (Triton X-100), or enzyme and EDTA or Enzyme, TritonX-100 and EDTA together. The concentration of Triton X-100 varied from 0.5-1ml/l and the concentration of EDTA was varied from 0.5-1g/l of Tris-HCl buffer. No mechanical agitation/shear was given during the flax retting process.

Figure 1: Left - The glass beaker with 0.5M Tris-HCl buffer and untreated chopped flax stems. Right: The water bath at 60°C, were flax retting takes place in closed glass beakers.

All the experiments were performed in duplo. After the incubation, the liquid was removed, the treated flax stems were washed 2-3 times in tap water in order to remove all the chemicals etc. The parts of the stems (4-6) were collected in a dry and clean test tube for Fried test (see 2.2.3 for more details). The stems samples were dried in oven at 150°C for 1hr. At the end

microscopic visualisation was made and flax retting was evaluated from 0-3 according to the Fried test.

2.2.3. Characterisation:

Fried Test: Fried Test [2] was carried out by 4-6 pieces of retted-stems, each approximately 5cm long. They are put into a test tube and 8ml of demineralised boiling water were poured over them. The test tube is then stoppered and vigorously shaken by means of a vortex mixer (TOP Mix FB15024, see Figure 2) for 30s to one minute. Subsequently, the boiled water was poured away. Then the 4-6 pieces of retted-stems were removed from the test tube carefully. All pieces of retted-stems were kept in the oven (Memmert Hot air oven) for drying at 150°C for 1 hr.

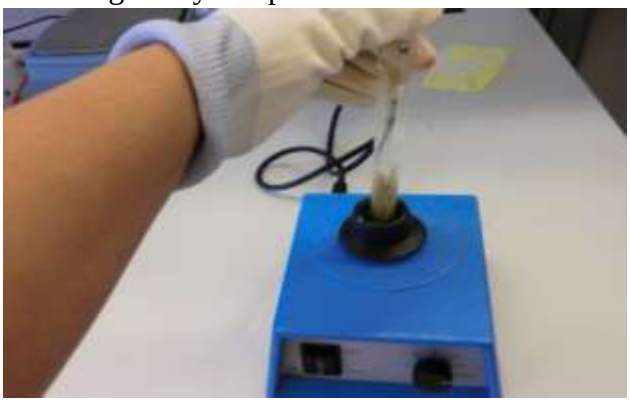

Figure 2: Fried test was carried out in glass tube with 4-6 treated stems boiling water, vortexes for 30-60 s.

Microscopic visualisation and retting evaluation: After drying, the retted stems were removed and subjected to microscopic examination. The microscope used was Dino-Lite (Digital Microscope-Pro) connected to the computer. The dried retted flax stems were kept under the microscope and the desired zoom/focus was adjusted for the best clarity. The degree of retting is evaluated from the microscopic photos on a scale of 0 to 3 and expressed as shown in Figure 3.

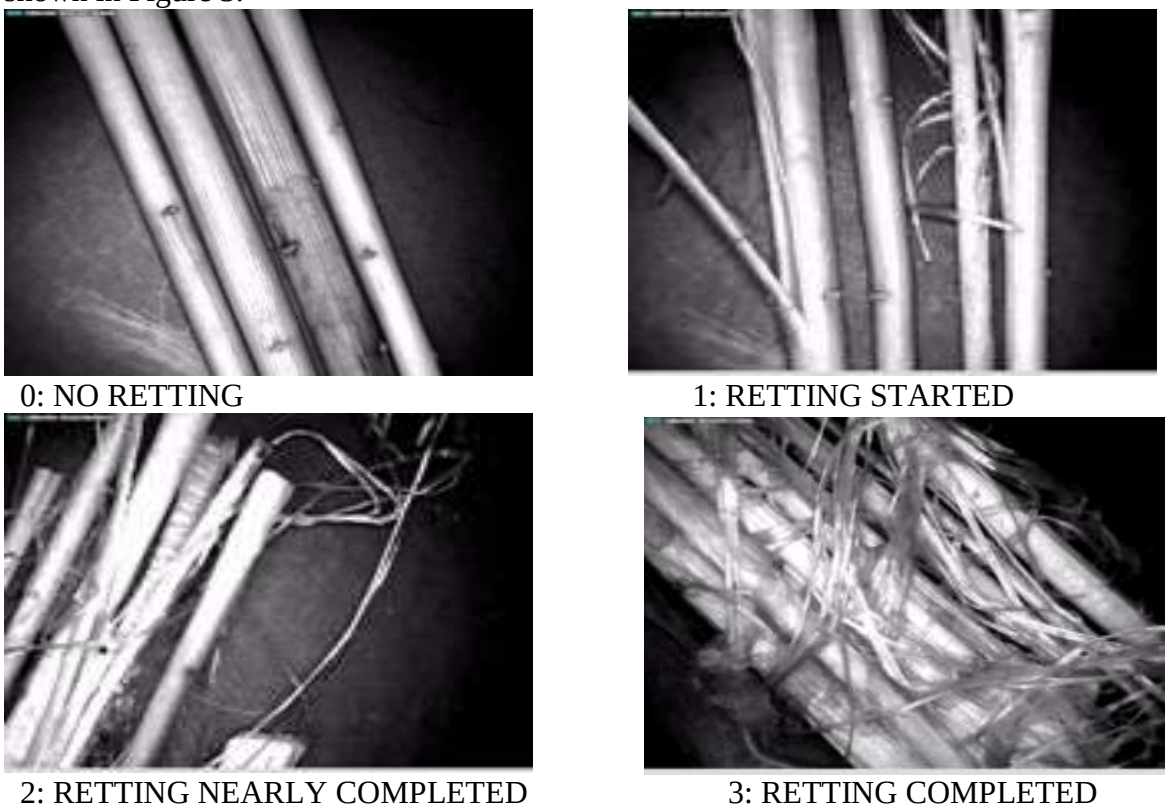

Figure 3: Microscopic visualisation and retting evaluation from 0-3.

3. RESULTS AND DISCUSSION

3.1 Optimisation of the process parameters: The bottom part of the flax stem is the most difficult part to ret owing to the complex and tight morphology. Therefore, process optimisation was conducted for the bottom part of the stem. Two different sets of optimal process conditions were determined as described below. A) 0.5M Tris-HCl buffer at pH 8.2 with 1.5 ml (413U) Scourzyme-L with 1:10 liquid ratio. The required time for flax retting is then 15 hours at 60°C. B) 0.5M Tris-HCl buffer at pH 8.2 with 0.5 g/L EDTA as chelating agent, 0.5 g/L non-ionic surfactant Triton X-100, 1.5 ml (413U) Scourzyme-L with 1:10 liquid ratio, the required retting time is then 9 hours at 60°C. The presence of a chelating agent and a surfactant apparently helps to accelerate flax retting.

3.2 Recycling experiments: Enzymes are bio-catalyst and are not consumed in the reaction. If the right conditions are maintained such as buffer, pH, temperature etc, the enzyme might be reused in the system. With this aim, experiments were carried using following process conditions 0.5 ml Tris HCl buffer at pH 8.2 with 0.5 g/l EDTA and 0.5 ml/l TritonX100 with 0.37 ml Scourzyme (103 U/g of flax) with 1:25 solid to liquid ratio. The experiments were conducted for 9 hrs at 60°C. Enzyme recycling experiments were conducted for 3 cycles. After each experiments, retted flax stems where removed and tested for the retting efficiency. The volume of supernatant was measured and brought up to total of 50ml by adding buffer solution each time before using for the next batch. The results suggest that all three recycling experiments show the complete enzymatic retting. In principle it is possible to recycle the enzyme which could help in lowering overall cost of enzymatic process for flax retting. However the further research needs to be carried out in order to optimise the process conditions using recycled enzymes, such as time, enzyme concentration etc and to study the effects on waste water composition and costs.

3.3 Scale up experiments: For uniform pilot scale experiments, the liquid ratio of 1:10 was not sufficient, due to uneven wetting of the stems. The liquid ratio was therefore enlarged to 1:25. Two sets of scaling up experiments were performed in a 1.5 litre bath and a 14 litre (560 g flax) bath. The increase in liquor ratio leads to better uniform retting results. The required enzyme concentration is reduced by 400% from 412 U/g (1.5 ml) of flax to 103 U/g (0.37 ml) of flax, while maintaining the 9 hrs retting time. The best experimental conditions for flax enzyme retting were found to be103 U/g of enzyme (0.37 ml), with a liquid ratio of 1:25 at pH 8.2 in a 0.5M Tris-HCl buffer containing 0.5 g/l EDTA and 1.0 g/l Triton X-100. It was decided to scale up this set of experiments with a factor of 280 (from a 50 ml beaker glass experiment to a 14 litre pilot scale experiment) while keeping other conditions similar. The only difference was using the whole flax stems to mimic the actual flax retting conditions. The pilot scale experiment was carried out in an open stainless steel vessel. The 560 g of flax stem were soaked into 14 litre of a Tris HCl buffer solution at 60°C (including 210 g of enzymes). The temperature at 60°C was maintained by keeping the reaction vessel inside the oven for 9 hrs. The COD and BOD values of the waste water were determined by sending the fresh supernatant (water water) to an analytical lab in Deventer, NL within 12 hrs after the experiments. The overall results in terms of flax retting and COD/BOD for the pilot scale (14l) experiment are summarized in Table 1.

It is clear from the Table 1, that a uniform flax retting of 3 has been achieved in the pilot scale experiments by utilising a higher liquid:solid ratio of 1:25 and a lower enzyme concentration (103 U/g of flax). The COD values are in order of 19300 mg/l and the BOD values are 988 mg/l.

Table 1: Flax retting and COD/BOD results for pilot scale experiment (14L)

Buffer (ml)	**Enzyme (ml)**	**Flax (gm)**	**Temp (°C)**	**Time (hrs)**	**Retting**	**COD (mg O_2/l)**	**BOD (mg O_2/l)**
13790	210	560gm (w with flowers)	60	9hrs	3	19300	988

3.4 Economic evaluation of the flax retting process

Based on the results of the experimental work, an industrial size enzymatic retting process was designed. This design allowed a rough cost estimation of the enzymatic retting process. The cost price was based upon the costs of chemicals, investments costs (depreciation), energy costs, man hours and waste water costs. Eventual drying and handling costs were not included in the calculations. For the waste water costs, the costs for disposing the waste water into the municipal sewage system in the Netherlands were used. The costs are expressed in € per kg of treated flax as a function of production scale (Table 2).

Table 2: Cost price analysis of the enzymatic retting process as a function of scale

Production in ton flax per year	Depreciation in €/kg	Chemicals in €/kg*	Heating in €/kg	Man hours in €/kg	Waste water in €/kg	Total costs in €/kg
876	0,028	0,76	0,04	0,11	0,98	1,92
1314	0,022	0,76	0,04	0,07	0,98	1,87
1752	0,018	0,76	0,04	0,06	0,98	1,85
2190	0,017	0,76	0,04	0,04	0,98	1,84
4380	0,014	0,76	0,04	0,02	0,98	1,81
6570	0,012	0,76	0,04	0,01	0,98	1,80
8760	0,012	0,76	0,04	0,01	0,98	1,80
10950	0,011	0,76	0,04	0,01	0,98	1,80
13140	0,011	0,76	0,04	0,01	0,98	1,80
17520	0,010	0,76	0,04	0,01	0,98	1,79
21900	0,010	0,76	0,04	0,00	0,98	1,79

***Of which enzyme costs are € 0,66 /kg**

From the table it is clear that the cost price of the enzymatic retting process is mainly determined by the price of the chemicals (i.e. the enzyme costs) and the waste water costs. The waste water costs are very high because of the very high COD (20000 mg O_2 per l) and nitrogen content ($N_{Kjeldahl}$ = 1500 mg/l) of the waste water after retting.

Based on information of the Dutch Flax agency (3), the price difference between high quality long fiber flax and low quality short fiber flax was estimated to be about € 1,20 per kg of fiber. As the aim of the enzymatic retting process is to enlarge the long fibers content, only part of this price difference is however available for the improved retting process, let's assume a maximum of € 0,60/kg of fiber. Two important conclusions can therefore be drawn from table 1. The first one is that the waste water costs are too high to make enzymatic retting in this set-up economically feasible. This figure can probably be much improved by anaerobic waste water treatment on site, also producing biogas for heating up the process water and/or the air in the drying process. However, leaving out the waste water costs, the table also shows that the enzyme costs are relatively high, leading to a total cost of at least € 0,66 per kg of flax stems (not flax fibers). Taking into consideration that the logistics and the consecutive drying process were also not included in the calculations, this makes it highly unlikely for the enzymatic flax retting process to be economically feasible. The only solution to improve this

would be enzyme recycling. However, more research to study this and also the consequences for the waste water composition is necessary.

4. CONCLUSIONS

A systematic approach has been adopted to design sustainable enzymatic flax retting process. Based of literature survey and initial experiments, alkaline pectinase (pectate lyase) has been selected as enzyme of choice for the flax retting. Supporting role of auxiliaries such as non-ionic surfactant and chelating agent has been established. The process conditions have been optimised in terms of pH, temp, incubation time, liquid to solid ratio etc. For lab scale experiments liquid to solid ratio of 1:10 is sufficient, however for larger volumes 1:25 LCR is required. Two sets of scaling up experiments were performed in 1.5 litre bath and 14 litre (560 g flax) baths. The increase in liquor ratio leads to better uniform retting results. The required enzyme concentration is reduced by 400% from 412 U/g (1.5 ml) of flax to 103 U/gm (0.37ml) of flax, while maintaining the 9 hrs retting time. The economic feasibility study shows that the cost of chemicals including enzymes makes this process expensive. The only solution is to recycle enzyme in the process. However more research is required to make this process industrially acceptable.

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