Saimaa University Of Applied Sciences
Unit Of Technology,Imatra
Degree programme in Chemical Engineering

Niraj Panjiyar

APPLICATIONS OF XYLANASE, LACCASE ENZYME AND HIGH POWER ULTRASOUND ON DIFFERENT NON-WOOD PLANTS

Bachelor´s Thesis, 2011

Abstract

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Supervisors: Mr Levente Csoka, DrTech, Assistant Professor, University of West Hungary and Mr Jarkko Männysalo,MSc ,Senior Lecturer,Saimaa UAS,Finland

The purpose of this bachelor`s thesis was to analyze the effect of properly applied microbial enzymes on non-wood plants delignification, improved fibre flexibility, fibrillation, removal of xylan and facilitated contaminant. The enzyme plays important role in digestion of fibres and removes lignin content of pulp.

In the experimental part, two different non-woody plants were experimented: flax, straw.

The enzymes Laccase and Xylanase were used. The amount of enzyme was tested in four part: 0.006g,0.042g,0.048g,0,003g.The experiment was carried out with this different amount of enzyme using flax and straw(two non-wood plant).The different parameter were used to analyze their results : change in temperature, concentration of enzyme, change of ultrasound time and change in Ph level.

The aim of work was to remove lignin content from non-wood plants with use of enzyme and ultrasound treatment .The parameter like changes in temperature, concentration of enzymes, ultrasound time and pH values were used in this experiment. These play an important role in separation and digestion of much better fibres.

Keywords:non-wood plants,enzymes

CONTENTS

1. INTRODUCTION

The aim of this thesis work is to show dependence of enzyme efficiency on the different pulp raw materials and then on combination with high-power ultrasound assisted pulping.

In the theory part, physical part of non-wood plants and their importance in future end users are explained. The enzymes: Laccase and Xylanase and their importance to these non-wood plants are explained. Additionally, different pulping methods are explained. The different parameters with their results are analyzed.

The experimental part consists of the laboratory measurements carried out in the facilities of University of West Hungary In Sopron.

2 NON WOOD PLANT

2.1 General

Generally, nonwood plant fiber pulps can be grouped into two broad categories:
Common non-woods or hardwood substitutes such as cereal straws, sugarcane bagasse, bamboo (shorter fiber species), reeds and grasses, esparto, kenaf (whole stalk or core fiber), corn stalks, sorghum stalks etc.
specialty non-woods or softwood substitutes such as cotton staple and linters; flax, hemp and kenaf bast fibers; sisal; abaca; bamboo (longer fiber species); hesperaloe etc.Some of uses of non wood plant are as fallows;printing and writing papers, linerboard, corrugating medium, newsprint, tissue, specialty papers.

Typically, common nonwood pulps or hardwood substitutes are produced in integrated pulp and paper mills, and softwood kraft or sulfite pulp is added to provide the strength requirements to the paper.

However, specialty nonwood pulp may be used instead of softwood kraft or sulfite pulp thus producing a 100% nonwood paper. And, in some cases, wastepaper pulp may be blended in the furnish. The nonwood portion of the furnish typically varies from 20 to 90% and can be even up to 100% dependingon the paper grade and required quality. The possible combinations are endless and can be adjusted tomeet market requirements.

Furthermore, it is possible to add small quantities (up to 20 - 30%) of common nonwood pulps toprimarily woodpulp-based papers without impairing paper properties or paper machine runnability. This provides wood-based mills which are hardwood deficient but located within a region with available nonwood fiber resources such as cereal straw or corn stalks with the option of adding-on a nonwood pulping line to supplement their fiber requirements. some of non wood plant are show in Table 1 . (Non wood plant fiber uses in paper making,Robert w.Hurter,2001)

2.1.2 History of Paper and Importance of Non-Wood Plant Fibers

When we think of raw materials for making pulp and paper, we normally think of wood. This is because, in North America and most other developed regions, wood at present constitutes almost 99%of total fibrous raw material used for pulp, paperboard, and reconstituted panel board. Nevertheless, even in these regions, the highest grades of paper are still made from non-wood plant fibers, and in regions, not so well endowed with forests, many other paper grades as well. For worldwide papermaking, therefore non-wood fiber raw materials and the techniques for making paper from them are of importance. (Hamilton and Leopold ,1993, p1)

Wood as a paper making raw material is a relative new comer; for nine-tenths of its history, paper was made almost exclusively from non-wood plant fibers. The first true paper is credited to Ts as Lun in 105 A.D.in China, and he apparently made it from textiles wastes, old rags, and used fish nets, i.e. the fibers of true hemp (*Cannabis sativa*) and China grass(ramie,*boehmeria nivea*).Because of the processing that these fibers had already received in the textile-making process, they could be prepared for papermaking by little more than beating which was done by macerating them in a mortar.

Ts´ai Lun`s paper soon found a market niche as a writing material between woven silk, which was more expensive, and split bamboo, which was less convenient.It also found many other uses,eg.as currency, toilet paper, and glazing for windows and lanterns. The demand for it became so great that a search soon began for additional fibrous raw materials. The first suitable raw fiber the Chinese found-i.e. straight from the plant-seems to have been the inner bark of the paper mulberry(*Broussonetia papyrifera*)[3,4]. This needed to be first separated from outer bark, and then soaked in alkaline solution of lime or wood ash, before being macerated. Another raw fiber used was bamboo, which was needed an even longer soaking, up to several months[1,5].These procedures represented the beginnings of the technique of pulping, as distinguished from that of making pulp into paper.

During the next 600 years, the technology of making pulp and paper spread east as far as Japan, south as far Asian, and west as far as Chinese Turkestan. In Turkestan, in 751 A.D., The technology was acquired by the Arabs, who transmitted it farther westward over their trade routes from Samarkand to Bagdad(793),Damascus, Cairo(900),and Fez(1100)[6]. Thence it spread to Spain(1151),France(1348),and Germany(1390)[1]. In regions where paper mulberry, bamboo and china grass were not available, they were replaced as raw material by linen and cotton rags. (Hamilton and Leopold , 1993,p 112)

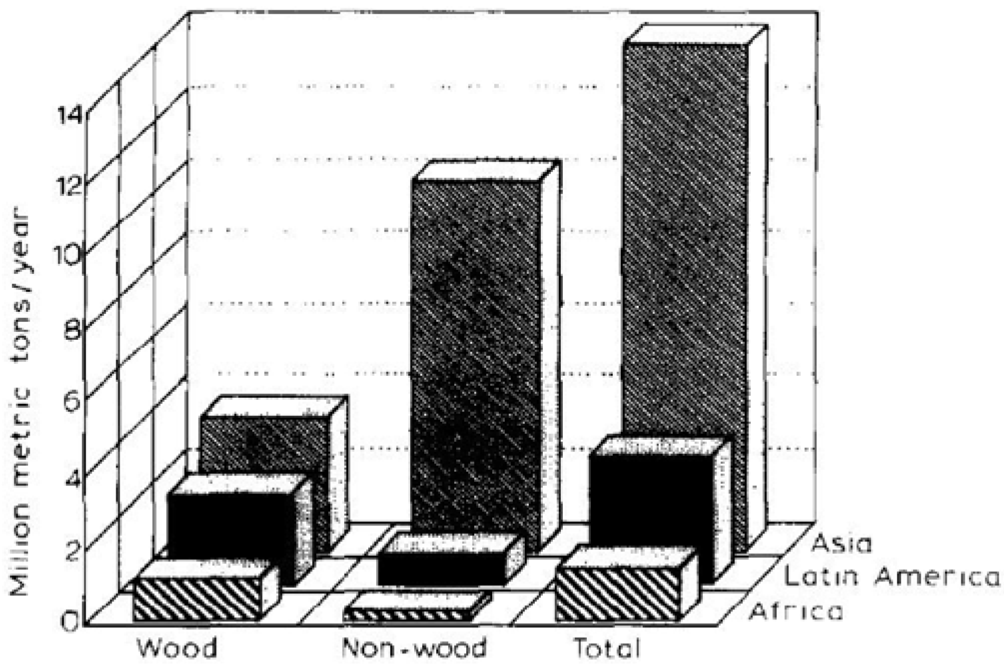

Fig 1.Pulp Production based on woods & non-wood fibrous raw-materials in developing regions in 1989 (FAO Year Book, Forest Product 1989)

2.2Flax

Flax (also known as **common flax** or **linseed**) (binomial name: ***Linum usitatissimum***) is herbaceous annual plant grown for fiber used in the production of linen for seed used in the production of linseed oil. When planted densely for fiber production of linseed oil, the plant stem grow to a height of about 0.9-1.2 m, with the branches all at the top. the stems have a diameter of 0.25-0.5cm.When grown for seed, the plant is much shorter and begins to branch continuously a short distance above grade.

The stems of flax plant have a woody core(known as the “ shive ”),with a hollow center . The woody core constitutes approximately 70% of the stem. It has very short fibers and produces pulp not unlike a rather weak hardwood pulp. The long fibers used for the production of linen and for papermaking are located in the bark of stems. (Hamilton and Leopold, M.J.Kocurek,1993 ,p 4.)

1. flax (Wikipedia)

2.2.1 Producers

Flax is grown in many parts of the world, but top quality flax is primarily grown in Western Europe. In very recent years bulk linen production has moved to Eastern Europe and China, but high quality fabrics are still confined to niche producers in Ireland, Italy and Belgium. Also countries including Poland, Austria, Belgium, France, Germany, Denmark, Lithuania, Latvia, the Netherlands, Italy, Spain, Switzerland, Britain and some parts of India. (Wikipedia)

2.2.2 Use of Flax

Because of its long slender fibers, flax pulping is ideal for the production of thin strong papers, such as cigarette papers, airmail papers, bible papers, and light-weight bond papers. Also, it is well suited for the production of currency papers and permanent record bond papers where strength, scuff resistance, and permanence are required.

Almost all of flax plant pulp produced from seed flax straw tow is used for the production of cigarette papers, as a substantial proportion of short fiber pulp can be tolerated as part of the furnish. Flax pulp from textile flax tow is more commonly used for the production of currency papers, high-quality permanent record papers (usually in combination with cotton pulp). (Hamilton and Leopold ,1993,p 114.)

2.3 Straw

Cereal straws are an important source of raw material for pulp materials for pulp and papermaking in many countries of the world where pulp wood availability is extremely limited. Straw is know as the oldest papermaking material and remained a major source of fiber in Europe and North America until the wood pulp industry was established during the latter part of the last century. Straw pulping gradually declined with increasing labor cost for collection, transportation, handling, and storage. (Hamilton and Leopold, 1993 p 82.)

2. Straw (Wikipedia)

2.3.1 Physical Structure and Chemical Properties

The stems or clumps of cereal straw are erect, elastic, generally tubular structures, separated at intervals by nodes, which occur as rated at intervals by nodes, which occur as vascular bundles crowded together and interlaced to form a strog diaphragm between the internodes. The rachis, or top portion of the stem to which seed is attached, is generally found with the straw. The leaf, starting at the node, from a sheath part way up the stem,leaf,sheath, and blade. Chaff consists of small broke pieces of stem, leaf sheath, and blade a log with various materials, such as seeds hulls(glumes).and bristles*(awns),which together with the seed and rachis form the head of the plant. Straws usually haves six internodes and grow 0,5 to 1,5 m tall, depending on the strain,climate,soil conditions. The internodes generally increase in length from base to apex of the straw, the top internodes being the longest. The culms wall thickness decreases from the base to the top of straw. (Hamilton and Leopold , 1993,p 83-84.)

2.3.2 Use of straw

As stated earlier, semichemical wheat straw pulp is used exclusively or in combination with waste paper to produce corrugating medium for box board manufacture. Other unbleached varieties, such as linerboard, test liners, M.G. imitation Kraft, and grocery bags, are produced with 60-65% straw pulp blended with long-fiber Kraft pulp and selected waste paper. Sack papers for the manufacture of multi-wall papers can be produced with 35-40%straw pulp content using the clupack extensible system. Grease-proof, glassine, toilet, and other household tissue can be produced containing 50-70% straw pulp. Straw pulp requires very little refining to prepare stock for various grades of writing, printing and poster paper. (Hamilton and Leopold, 1993, p 91.)

3. ENZYMES

Enzymes are proteins that catalyze (*i.e.*, increase or decrease the rates of) chemical reactions. In enzymatic reactions, the molecules at the beginning of the process are called substrates, and they are converted into different molecules, called the products. Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell.

Like all catalysts, enzymes work by lowering the activation energy($E_a^{\ddagger}$) for a reaction, thus dramatically increasing the rate of the reaction. As a result, products are formed faster and reactions reach their equilibrium state more rapidly. Most enzyme reaction rates are millions of times faster than those of comparable un-catalyzed reactions. As with all catalysts, enzymes are not consumed by the reactions they catalyze, nor do they alter the equilibrium of these reactions. However, enzymes do differ from most other catalysts by being much more specific.

Enzyme activity can be affected by other molecules. Inhibitors are molecules that decrease enzyme activity; activators are molecules that increase activity.

Many drugs and poisons are enzyme inhibitors. Activity is also affected by temperature, chemical environment (*e.g.*, pH), and the concentration of substrate.

Enzymes are widely used in industrial applications like in food processing companies (e.g in production of sugars from starch, such as in making high-fructose corn syrup. In baking, this catalyze breakdown of starch in the flour to sugar. Yeast fermentation of sugar produces the carbon dioxide that raises the dough), paper industries (enzymes degrade starch to lower viscosity, aiding sizing and coating paper. Xylanases reduce bleach required for decolorizing; celluloses smooth fibers, enhance water drainage, and promote ink removal; lipases reduce pitch and lignin-degrading enzymes remove lignin to soften paper). Brewing companies (then widely used in the brewing process to substitute for the natural enzymes found in barley). Biofuel Industry(thet are used to break down cellulose into sugars that can be fermented and use of lignin waste).Rubber industry(enzynes to generate oxygen from peroxide to convert latex into foam rubber),Photographic industry(to dissolve gelatin off scrap film, allowing recovery of its silver content. Molecular biology industries (used then manipulate DNA in genetic engineering, important in pharmacology, agriculture and medicine. They are also an essential for restriction digestion and the polymerase chain reaction. Molecular biology is also important in forensic science). Contact lenses cleaner Company (To remove proteins on contact lens to prevent infections). (Tutorvista,2010)

3.1 Xylanase

Xylanases are glycosidase (*O*-glycoside hydrolyses, EC 3.2.1.x) which catalyze the end hydrolysis of 1,4-β-D-xylosidic linkages in xylan. They are a widespread group of enzymes, involved in the production of xylose, a primary carbon source for cell metabolism and in plant cell infection by plant pathogens, and are produced by a plethora of organisms including bacteria, algae, fungi, protozoa, gastropods and anthropoids. First reported in 1955, they were originally termed pentosanases, and were recognized by the International Union of Biochemistry and Molecular Biology (IUBMB) in 1961 when they were assigned the enzyme code EC 3.2.1.8. Their official name is endo-1,4-β-xylanase, but commonly used synonymous terms include xylanase, endoxylanase, 1,4-β-D-xylan-xylanohydrolase, endo-1,4-β-D-xylanase, β-1,4-xylanase and β-xylanase.

In the present review, the diversity of xylanases, their substrate, action and function, their importance in industry, classification into families and adaptation to various extreme environments are discussed. Special emphasis is paid to the 'new' xylanase containing families, highlighting their similarities and differences to the better known family 10 and 11 members, as well as to the peculiarities and interests of these hitherto scantily reviewed enzymes. In addition, the adaptation strategies, characteristics and industrial potential of extremophilic xylanases will be discussed.

Xylanases are hydrolytic enzymes which randomly cleave the β 1,4 backbone of the complex plant cell wall polysaccharide xylan. Diverse forms of these enzymes exist, displaying varying folds, mechanisms of action, substrate specificities, hydrolytic activities (yields, rates and products) and physicochemical characteristic. (onlinelibrary, 2010)

Table 4 Application of Xylanase (onlinelibrary,2010)

Market	**Industry**	**Application**	**Function**
Food	Fruit and vegetable processing, brewing, wine production.	Fruit and vegetable juices, nectars and purees, oils (e.g., olive oil, corn oil) and wines	Improves maceration and juice clarification, reduces viscosity. Improves extraction yield and filtration, process performance and product quality.
	Baking	Dough and bakery	Improves elasticity and strength of the dough,

Market	Industry	Application	Function
		products	thereby allowing easier handling, larger loaf volumes and improved bread texture.
Feed	Animal feeds.	Monogastric (swine and poultry) and ruminant feeds	Decreases the content of non-starch polysaccharides, thereby reducing the intestinal viscosity and improving the utilization of proteins and starch. Improves animal performance, increases digestibility and nutritive value of poorly degradable feeds, e.g., barley and wheat.
Technical	Paper and pulp	Biobleaching of kraft pulps	Reduces chlorine consumption and toxic discharges.
		Bio-mechanical pulping	Facilitates the pulping process and reduces the use of mechanical pulping methods, hence reduces energy consumption.
		Bio-modification of fibers	Improves fibrillation and drainage properties of pulp, hence improving the process efficiency and the paper strength.

Market	Industry	Application	Function
		Bio-de-inking	Facilitates the de-inking process and reduces the use of alkali.
Starch		Starch-gluten separation	Reduces batter viscosity, improves gluten agglomeration and process efficiency.
Textiles		Retting of flax, jute, ramie, hemp, etc.	Enzymatic retting, reduces/replaces chemical retting methods.

3.2 Laccase

laccase is type of enzyme cupper containing polyphenol oxidase that was discovered in the exudates of the Japanese lacquer tree *Rhus verinicifera*(yoshida,1883) and sub-sequent was demonstrated as fungal enzyme as well as(Bertand,1986;Laborde,1896). At present there is only one bacterium,*Azospirillium lipoferum*,in which laccase-type phenol has been demonstrated (Givaudan *et.a.l*,1993). Laccase is one of small groups of enzymes called the large blue copper proteins or blue cupper oxidases.The other member of this group plant absorb ate oxidases and the mammalian plasma protein called ceruloplasmin. The blue oxidases have been intensively studied not least because they shared with terminal oxidases of aerobic respiration the ability to reduce molecular oxygen to water. It is therefore paradoxical that our knowledge of these proteins is so incomplete.

The laccase has also same kind of uses as xylanase. The most widely used field of laccase are food industry, pulp and paper industry, textile industry, Nanotechnology, other laccase applications like soil bioremediation, synthetic chemistry, cosmetics.(Sgmjournals,1994.)

4 DIFFERENT PULPING METHODS

The process where wood or other lignocellulose material is reduced to pulp is called pulping.Defibration can be accomplished chemically, mechanically or by comingling these methods. Pulps made from different raw materials and by different methods have unique properties. Different pulps are suited to particular products. Worldwide, chemical pulping is the predominant pulping method. In 2000, the chemical pulps had 77% share of all wood-based fiber material globally. (sixta 2006) . In Finland, however, mechanical pulps play a bigger role: in 1998,mechanical and semi-chemical pulps comprised 40.8% of Finland´s pulp production (Seppälä et al., 2005) .

Best strength properties are achieved with chemical pulp made from softwoods, whereas lowest values are achieved with grinding methods. Generally, bigger the proportion of long fibers in the pulp, better the properties. On the contrary, when there are more short fibers in the pulp, it will give better smoothness for paper. (Häggblom-Ahnger& komulainen 2005.)

4.1 Chemical Pulping

In the chemical pulping, lignin is dissolved through chemical reaction at elevated temperatures. In the pulping process, temperature can be high 170 degree Celsius. Usually the target is that 90% of lignin will be removed.Simultaniously, with lignin removal, significant parts of hemicelluloses are removed as well. The total fiber yield is typically from 45% to 55% .The yield varies to a larger extent, depending on the wood source and the pulping process applied. It is important to stop the chemical reactions at a point when the lignin content is suitably low, and acceptable yield can be still be achieved. (Sixta 2006.)

Chemical pulping processes can be divided into acid and alkaline processes. The most common alkaline process is the Kraft process and sulphite pulping is the most important acid process. At present, the Kraft process, is the predominant chemical pulping method. In Finland, for instance, the production of sulphite pulp has been stopped. Earlier, sulphite pulping was the leading method, but it has lost its position to the Kraft process due to several reasons: new techniques enabling efficient bleaching of Kraft pulp, environmental reasons, the Kraft process is applicable to bigger number of wood species. (Gullichsen & Fogelholm 2000.)

In the Kraft pulping process, wood is debarked and chipped. Chips are then screened to ensure suitable sizes and dimensions. The accept chips are fed to the digester. The chips are then streamed and subsequently the digester is filled with warm cooking liquor to submerge the chips. The constituents of the cooking liquor are white liquor and spent black liquor from a preceding cook. The digester contents are heated by direct steam or by indirect heating in a liquor/steam heat exchanger to 160 -170 degrees Celsius. The desired cooking temperature is maintained until the target degree of delignification is reached .After cooking the pulp is washed and screened. The spent liquor is recovered in a washing system. In the screening operations, incompletely delignified residues of wood, which are not broken down to the fibers during the blow from digester, are separated from suspensions. In most of the Kraft pulp mills, pulp is also bleached. (Gullichsen &Fogelholm 2000.)

4.2 Mechanical pulping

In the mechanical pulping, wood fibers are liberated by mechanical means only. Mechanical pulp manufacturing process can roughly be divided in two categories; the refining process and the ground wood process. The main mechanical pulps are called stone ground wood (SGW), refiner mechanical pulp (RMP) and thermo-mechanical pulp (TMP). (Karlsson, 2006) Mechanical pulping processes offer certain advantages compared to the chemical processes. Capital costs are lower and furthermore, the process is less complicated. The yield in mechanical pulping can be over 95% .Generally mechanical pulps are produced at the paper mill. Mechanical pulping processes also have their drawbacks. They require high-quality wood raw materials. Additionally; the electric energy consumption is considerably high. (Sundhlom 1999, 20.)

4.2.1 Stone ground wood pulping and pressurized ground wood pulping

The ground wood process (SGW) is the oldest method of mechanical pulping. In it, a block of wood is pressed across against a roughened, revolving grindstone. Typical diameter of the grindstone is 1.5 m and typical rotation speed from 240 to 300 rpm.Fibers, are in a way, torn out of the wood. Mechanical energy converts partly to heat participates in softening the lignin binding the fibers together and also assists in opening the bonds between fibres.The process is relatively simple, but a uniform quality may be hard to achieve.Pressurised ground wood(PGW) is produced by a similar type of method. In PGW-process, the grinder is pressurized with steam. Increased pressure improves the fiber separation and physical properties of the pulp. Energy consumption is lower than in SGW-process. (Karlsson 2006 .)

4.2.2 Refiner mechanical pulping and thermo mechanical pulping

Refiner mechanical pulp is manufactured from wood chips in a disc refiner. Normally, it is made from softwood chips, which are pumped through a narrow opening between rotating metal disc. The diameter of discs is typically 1.5 m.Orginally the defibration took place at atmospheric pressure, but later refining under increased steam pressure was introduced. Normally, the process involves two refining stages in series.Thermomechical pulp (TMP) is nowadays the most common type of mechanical pulp. It has been modified from RMP. In TMP-process, the raw material is steamed before and during refining. The steaming softens the chips resulting in higher percentage of long fibers and fewer shives in the produce pulp. (Karlsson 2006.)

4.3 Chemical Pre-Treatments

It is possible to modify the thermo mechanical pulp with small amounts of chemicals, such as sodium sulphite (Na_2so_3).The modification is carried out prior to defibration in refiner. The pulp produced with this method is called chemical mechanical pulp (CMP) or chemical thermo mechanical pulp (CTMP).These methods can be used in refining hard woods (birch and aspen) as well. If these pulps are bleached, they are called bleached chemical mechanical pulp (BCMP) and bleached chemical thermo mechanical pulp (BCTMP). BCTMP is often produced in non-integrated mills. (Karlsson 2006.)

6 Materials and Method

following materials and methods were used in the study:

Precisa Balance	-Precisa 125 A (PAG Oerkikon AG ,Zurich) ,made in Switzerland .
Fast Dryer	-Mls50-3 (Kern and John GmbH),product of Germany .
Water Bath	-Memmert Company, made in Germany .

Spectrophotometer--S2000uv/vis (WPA,Cambridge) ,product of UK was used.280 nm and 480 nm absorbance was used because it gives clear view of graph in this range and flow was not high.

pH Meter	-Hi 8314 membrane PH Meter(Hanna Instruments)
Ultrasound	-Horn type equipment operating at 20 kh and 150 watt power.19.2mm diameter of horn .
Buffer Solution	-Citric acid/Sodium Hydroxide /Hydrogen Chloride) color red traceable to SRM from NIST and PTB .

RESULTS

The results cannot be published because overall research is unfinished, only some results were done by me. Hopefully, when all works are finished then it will be published later on along with mine results.

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