Preliminary Study

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PRELIMINARY STUDY OF DESIGN FOR MEDICINAL WASTE DETOXIFICATION THROUGH ENVIRONMENTALLY FRIENDLY REACTION BY UTILIZATION OF *SACCHAROMYCES CEREVISIAE*-BASED LOCAL TAPE YEAST

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ABSTRACT

The emerging pharmaceutical industry supports public health because it supports the production of needed medicines. However, this development is also marked by the presence of waste that still contains chemically active ingredients, and needs to consider about to be managed properly that needs to be reduced or even eliminated. One of the methods used in this preliminary study is the effort to eliminate activities with a reductive process that is environmentally friendly compared to the oxidative-degradation process that is commonly used. Diclofenac and ibuprofen are compounds selected in the design. In this preliminary study, compounds were selected as a model of compounds with active functional groups namely aromatics, carboxylates, and halogens. This study was designed with a physical and chemical approach using Indonesian local tape yeast containing stable *Saccharomyces cerevisiae* namely yeasts that have the potential to change the organic function groups of the sample.

Keywords: *Saccharomyces Cerevisiae, diclofenac, ibuprofen*

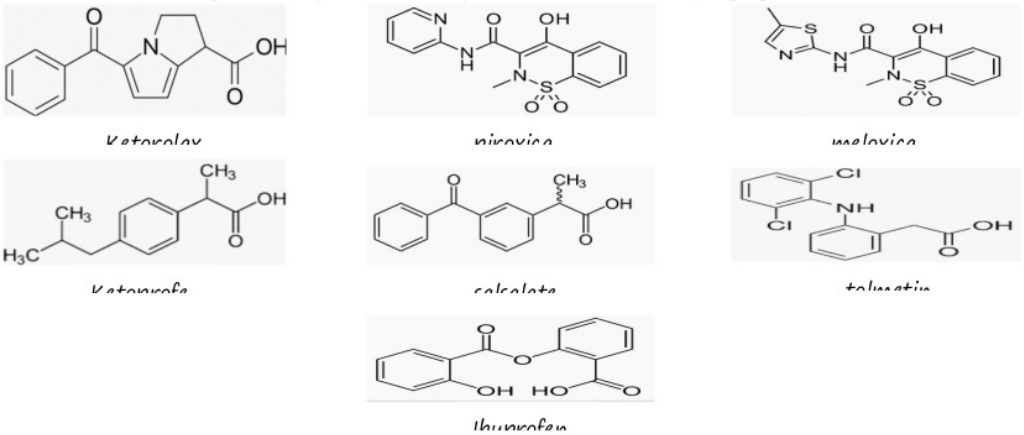
INTRODUCTION

The pharmaceutical industry is growing rapidly due to the need for drugs that are needed. The development of these technologies also produces other unavoidable materials, one of which is the environmental aspect. The industrial world such as the pharmaceutical industry produces chemical products which in the process are the stages of unavoidable important chemical reactions is the presence of waste materials that still contain chemicals. This waste material is still very potential as a material that may disrupt environmental harmony in terms of chemical aspects. For this reason, research in the field of environmental chemistry is very important to always continue to improve, namely the ability to reduce the potential and even eliminate chemicals that are categorized as hazardous and toxic. Today's chemists carry out an important mission in the 21st century, namely that they must actively participate in contributing ideas, initiatives, and actual activities that protect the environment, or can be termed as chemists who are at the same time able to create environmentally friendly processes (Green chemistry). In this research, it will be developed and tried out in a practical laboratory design of ways of thinking and applying on an environmentally friendly basis, i.e. the toxic potential of pharmaceutical industry waste materials can be transformed into reduced materials and even toxic properties are lost so that they become safer for the environment. The approach that will be used is to understand the chemical reaction by changing the functional groups of carbonyl compounds that are toxic in the waste into alcohol functional groups with the help of cheap and stable microorganisms that have been widely used in Indonesian society, namely yeasts which are known as the *Saccharomyces cerevisiae* microbes. These microbes have important properties namely the presence of enzymes belonging to the *reductase enzyme* group, which is expected to serve as a potentially good detoxification agent.

LITERATURE REVIEW

The pharmaceutical industry, which produces medicinal ingredients, has also received attention for its waste containing these medicinal ingredients. The pharmaceutical industry waste is generally in the form of liquid waste in which dissolved organic materials are found. One example of a medicinal ingredient is a class of *Nonsteroidal Anti-inflammatory Drugs (NSAIDs),* or also known as inflammatory pain medications. The active components in this medicinal ingredient are included in the class of carbonyl compounds as ketone compounds. Examples of ketone-based drugs include commercial products: piroxicam (feldene) meloxicam (mobic vivlodex) ketoprofen (orudis, ketoprofen ER, oruvail, actron), nabumetone (relafen), tolmetin (tolmetin sodium, tolectin), salsalate (disalcidal), ketorolac (toradol), and mefenamic acid (ponstel).

Figure 1. Examples of active compounds of anti-inflammatory drug ingredients



Some examples of anti-inflammatory drugs that have been used in society today with trade names are as varied as the following examples:

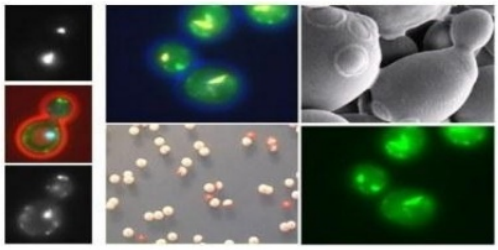
Figure 2. Examples of anti-inflammatory drugs in the market

The compounds mentioned above are known as anti-inflammatory drugs (NSAIDs). This medicine can be an alternative but at very low doses. This can reduce inflammation and pain, but with more serious side effects than acetaminophen. Long-term use can cause irritation to the stomach, gastric, and kidneys and increase the risk of heart disease and stroke. In addition, NSAID drugs such as aspirin and ibuprofen should only be used within ten days. In addition, available naxproven can fight pain and inflammation without the risk of causing heart disease. The problem is the side effects include indigestion, headaches, dizziness, and drowsiness. Diclofenac is also available in oral form (drinking) and topical (topical, external medicine) to reduce pain due to calcification of joints. It is known that this carbonyl structured compound has reactive properties so that if these compounds are present in waste such as sourced from pharmaceutical industry wastes, they need to be watched out and should be monitored properly. The waste containing active compounds will react with the environment chemistry so that it disrupts the ecosystem and stability and normal conditions, which in thetime fit to the current long-term this condition is detrimental to the environmental system. Therefore, it is considered important to design chemical steps that are at the same time fit the current conditions that are always supporting environmentally friendly processes, away from works that are detrimental to natural ecosystems. This effort can be found as a starting point for research aimed at detoxifying waste materials in a safe and environmentally friendly manner (*Green Chemistry*). For this reason, to overcome the potential danger of active ingredients of these drug, safe, inexpensive, and simple methods for preventing this potential need to be considered. One important chemical method is to change the reactive nature of the carbonyl active groups. Various studies have been developed, and one of the interesting focuses is eliminating the chemical reactive nature by being able to turn it into a less reactive group through oxidation, or vice versa, through a safer reduction reaction. It is known that oxidation reactions can affect the byproducts that are still reactive, conversely, if, through a reduction reaction, the results obtained are safer. However, it is known that reductive processes in organic chemistry require expensive reagents, so the industry actually rarely applies them. For this reason, in this study, an alternative method is found to change the carbonyl group in waste materials through reductive conditions but with a cheap and simple reducing agent. Therefore, in this preliminary study, yeasts containing stable Saccharomyces cerevisiae microbes were selected as ingredients to eliminate the reactive nature of the waste of discarded medicinal materials.

Yeast is a starter for making fermented Fermented black glutinous rice or cassava. In this yeast, microorganisms are found that can convert carbohydrates into simple sugars which are then converted into alcohol. This single-celled yeast is the eukaryotic type and multiplies by dividing itself. Unlike bacteria, yeast has a larger cell size, has organs, has a cell nucleus membrane, and DNA is localized in chromosomes in the cell nucleus. Yeast is a unicellular fungus and can be dimorphistic, which has two phases in its life cycle depending on environmental conditions, namely the hyphal phase (forming mycelium) and the yeast phase

(forming a single cell).

Figure 3. *Saccharomyces cerevisiae* microbes



Saccharomyces cerevisiae including an *Ascomycetes* type of yeast which contains a lot of protein, carbohydrates, and fat so that it can be consumed by humans and animals to supplement their daily nutritional needs. Saccharomyces cerevisiae also contains vitamins, especially vitamin B complex. *Saccharomyces cerevisiae* is easily digested and consumed. *Saccharomyces cerevisiae* yeast has a long history in the fermentation industry, the reason for its ability to produce alcohol makes the Saccharomyces cerevisiae is called as a safe microorganism. Yeasts that frequently used in making fermented ingredients are yeast with various trade names. Yeasts are generally round flat with a diameter of 4-6cm and a thickness of 0.5cm. so in these yeasts, there are microorganisms that can convert carbohydrates (starch) into simple sugars (glucose) which are then converted again into alcohol. Some types of microorganisms found in yeasts are *Chlamydomucor oryzae, Rhizopus oryzae, Mucor sp, Candida sp,* and *Saccharomyces cerevisiae*.

Fermented ingredient yeasts are made from a mixture of rice and spices, in general, yeasts contain various types of microorganisms from the mold group. Examples of local yeast products on the market include the following figure.

Figure 4. Commercial yeasts

It is known that the chemical aspects of synthesis have used a lot of yeast as biocatalysts because, in yeast cells, there is an enzyme system that is classified as a reductase enzyme. This system is supported by the biochemical content known as NADH that serves as bioreduction. As the following figure is shown, in the cell is found an enzyme system that can facilitate the presence of biological reagents as a very good reducing agent, NADH. NADH is formed from glucose starting material and is used for the formation of ethanol compounds. That is why saccharomyces sp. is very effective as a catalyst for alcohol formation which in practice has a good economic value, namely making fermented cassava or black glutinous rice.

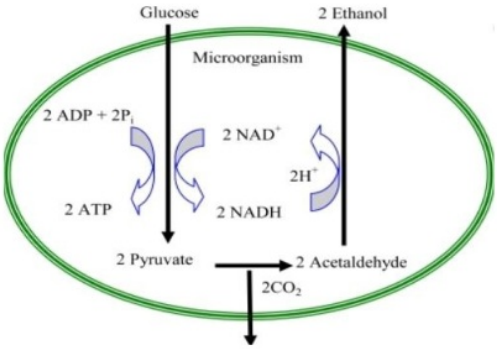


Figure 5. Chemical reaction system that occurs in microbial cells

In the latest research, NADH is developed as a catalyst to be able to convert carbonyl groups in medicinal materials into alcohol groups that stereochemically they become stereoselective, i.e. forming specific chiral alcohol compounds that are only one of the absolute (R) or (S) configurations.

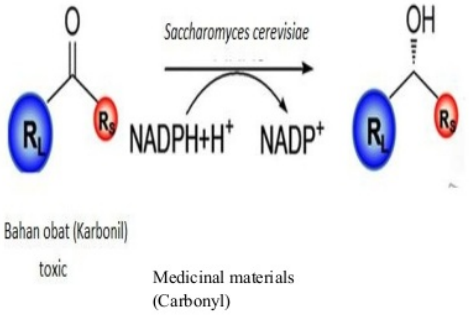


Figure 6. The principle of bioreduction of carbonyl compounds by Saccharomyces sp.

It is well known that microbes as a complete cell system facilitating the presence of important biological reagents such as NADH, cofactor compounds, complete media as shown below. The biological reagents will simultaneously change the chemical system of the initial substrate into a

specific product, as well as the formation of side products.

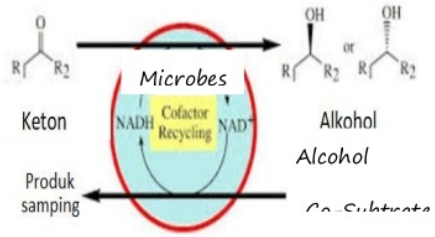


Figure 7. Bioreduction process using microbes

In this preliminary study, saccharomyce yeasts will be tested for their ability to reduce the carbonyl ketone model compound namely acetophenone, and it is hoped that with the success of this research, more detailed and advanced pilot scale design will be applied as an important part of pharmaceutical waste treatment in the future. This reaction can last under simple conditions, room temperature, and takes place in the aqueous solvent. The results of the product analysis are known and it can be concluded that it will produce stereoselective alcohol, so it is known as the ability of yeasts to convert the krokiral chemistry namely the carbonyl group converted into a specific alcohol group (i.e. chiral alcohol).

METHODS

Preparation of Saccharomyce cerevisiae yeasts as an adsorbent.

Yeasts are obtained from the local market. Furthermore, 10g of yeasts were added with 50ml of 0.1 N hydrochloric acid stirred and left for 1 hour. This treatment aims to stabilize and activate the functional groups as part of its activity aspects. This yeast product is then rinsed with distilled water until it is neutral again, and aerated, mashed, and stored in a desiccator before use.

Characterization of *Saccharomyces cerevisiae* Yeasts

Characterization of the results of yeast treatment was measured using FT-IR by using KBr with a yeast ratio of about 0.01%, and measurements using Particle Size Analyzer (PSA).

RESULTS AND DISCUSSION

Physical Yeast Testing (Physical Approach)

Measurement of adsorbent properties and pH variation test

A sample solution of diclofenac and ibuprofen was prepared by 0.1g in 100 mL distilled water, as well as 2g yeasts, put in Erlenmeyer, and stirred for 3 hours. Then filtered, the filtrate obtained was taken by 5mL to measure absorbency at 340nm wavelength for diclofenac samples and measured at 224 nm wavelength for ibuprofen. In addition, a standard solution was prepared as a standard graph. The capacity as a saccharomyces yeast adsorbent for samples was done by measuring the absorbent value and was calculated using the standard graph provided. An analysis of the adsorbent properties was also prepared with variations in pH, namely the pH 3.0, pH models 1, and pH 12.0.

Biomodification Testing Preparation

A biomodification of diclofenac and ibuprofen samples was prepared as follows: a number of each sample was prepared as a solution in distilled water. Saccharomyces cerevisiae yeast growth media composed of glucose (0.4g/100mL), NaCl 0.3g/100 mL), peptone (0.5g/100 mL), put in 500 mL round flask, added by saccharomyce yeasts (0.1g/100 mL), this mixture was left at room temperature and shaken. After 3 days, the mixture was filtered; the filtrate obtained was collected and extracted with ethyl acetate. A chemical analysis conducted was Product identification by thin-layer chromatography (TLC). A concentration of 10 ppm of acetophenone compound and its bioreduction product was prepared by 10mL each. A developer solution for chromatography was prepared by 10mL from hexane: ethyl acetate ratio (4:1) mixing placed in a 50 mL glacier, and immediately covered with a watch glass, and left for 10 minutes to saturate the conditions. The 2 x 4cm silica-gel made in the form of thin-layer chromatography (TLC) pieces was dipped in each sample of acetophenone and the product above, and then the TLC was put in the saturated glass beaker container. The chromatography is terminated after the developer solution reached the TLC and then, the TLC was immediately lifted and dried shortly by aerated manner. Chromatographic results were visually analyzed with the help of UV lamps and documented (photos). The created spots were observed and each Rf value was calculated for data.

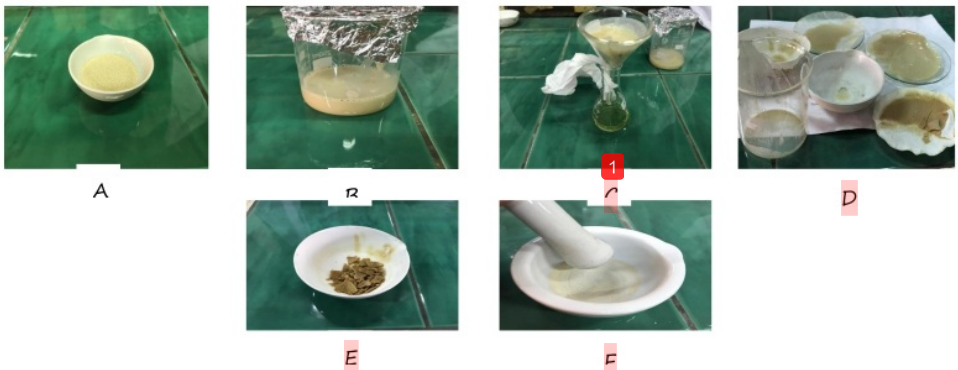


Figure 8. Pre-treatment of Saccharomyces cerevisiae yeast with 1 N HCl

Remarks (A) Yeasts; (B) Addition of 1N HCl for 1 hour; (C) Separation; (D) Activated yeasts; (E) Dry yeast as a result of acid treatment;

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(F) Refined yeasts

FT-IR Testing

FT-IR analysis aimed at determining the functional groups of organic compounds. It was known that yeasts were a formulation containing Saccharomyces sp. microbes. These microbes contained an enzyme known as amylase, although it was known to contain other enzymes. The analysis results were explained as shown below.

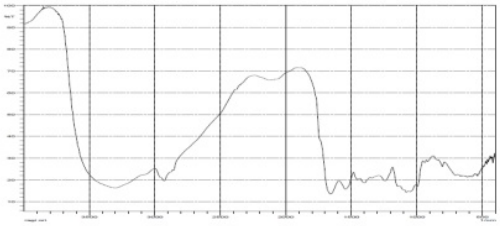


Figure 9. FT-IR spectrum of Saccharomyces yeast before it was used as an adsorbent

FT-IR spectrum of Saccharomyces yeast that was already activated by acidification showed that data in the area of 3448cm-1 in the form of broad absorption correlated with overlapping -NH and -OH. While the peak in the area of 2923cm-1 was representative of the C-H group. The peak at 1645 cm-1 was the deformed NH group, which might be an amide group. The peaks in the area of 1541cm-1 indicated the amide group.

When this data was compared with the results of the FT-IR analysis of Saccharomyces yeast which was already used for the test of absorption of diclofenac samples, it could be discussed that there were no significant chemical changes.

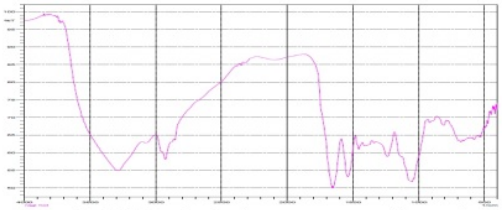


Figure 10. FT-IR Testing Saccharomyces yeast after used as an adsorbent

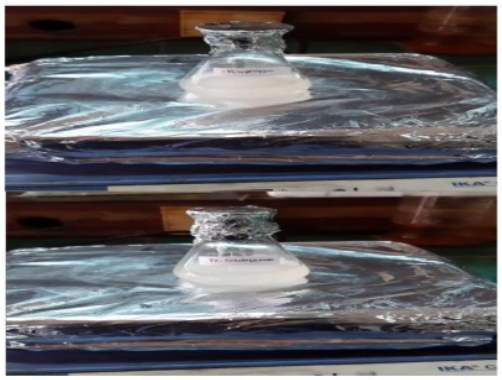
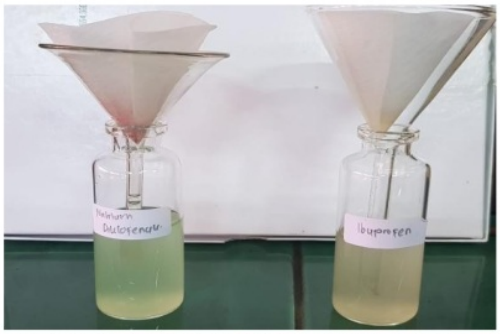


Figure 11. Testing of *Saccharomyces cerevisiae* yeasts for

However, there were other changes in the data, namely a slight decrease in absorbent values in the absorption area of 341cm^{-1} (-OH and -NH), and in the peak area of 1639cm^{-1} (-NH).

Chemical Testing (Chemical Approach)

The yeast used in this study was a local product, and it was known that this yeast was commonly used for fermentation, which was used for making fermented cassava raw materials. Yeast that was a solid material that contained Saccharomyces species microbes that commonly used was Saccharomyces cerevisiae, which had a specific activity as a biocatalyst because it contained enzymes that could convert carbohydrates into simple sugars. However, from the emerging research, it was already informed that yeast might be used in addition to the hydrolysis of starch. It was known that yeast containing microbes had specific enzymes, so



several studies produced data that yeast might be used for reduction, transesterification,

biomodification of sodium diclofenac and ibuprofen

Figure 12. Preparation of a solution of diclofenac Sodium and ibuprofen separated from yeast materials.

Figure 13. Solutions of diclofenac sodium and ibuprofen samples were ready for chemical testing.

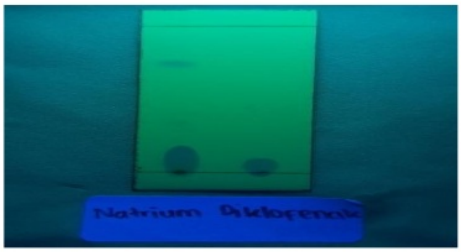


Figure 14. Thin Layer Chromatography Test (TLC) of Sodium diclofenac. Remarks: Left spot: Na-diclofenac reaction results with yeasts Right spot: Na-diclofenac

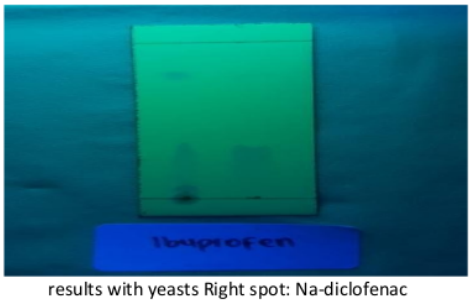
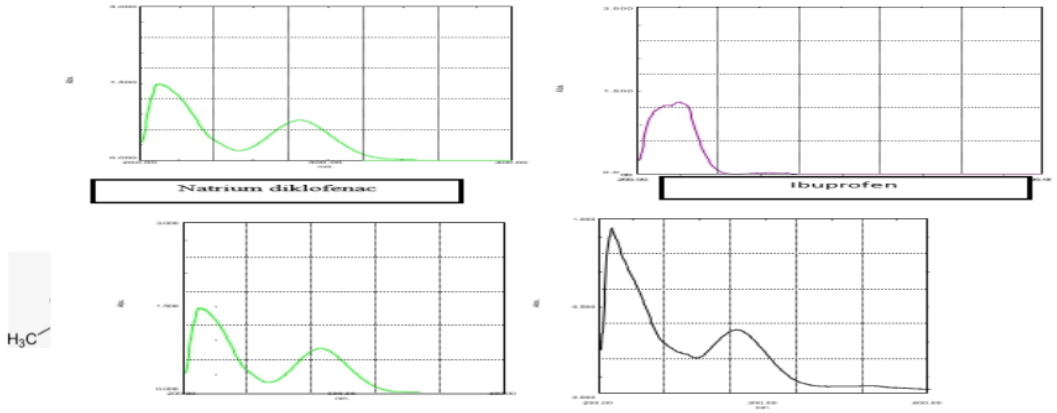


Figure 15. Thin Layer Chromatography Test (TLC) for Ibuprofen Remarks: Left spot Ibuprofen reaction results with yeasts Right spot: Ibuprofen Measurement using a UV-Vis spectrophotometer

The diclofenac and ibuprofen samples were organic compounds composed of carbon bonds so that they had a character that could be observed using a UV-Visible spectrophotometer. The UV-Vis spectrophotometer was useful for observing organic compounds that had functional groups supporting the character of a chromophore, which was composed by the existence of double bonds, which were supported in the carbon atoms.

As in the diclofenac compound, the chromophore group was supported by conjugated double bonds in its aromatic structure and carboxylate groups. Whereas, in the sample, the ibuprofen compound was composed of two aromatic units, the carboxylic group, and the amine group. The measurement results of the two samples were shown below.



The diclofenac sodium compound had a maximum absorption at a wavelength of 257 nm, whereas ibuprofen at a wavelength of 227 nm. A measurement using UV-Vis could be used as a parameter of qualitative and quantitative analysis so that a decrease in sample content due to chemical events could be observed with this analysis. In this study, the chemical treatment with *Saccharomyces cerevisiae* yeasts from local materials on samples of this model, changes in biomodification were observed using UV-Vis, and the results obtained were as follows:

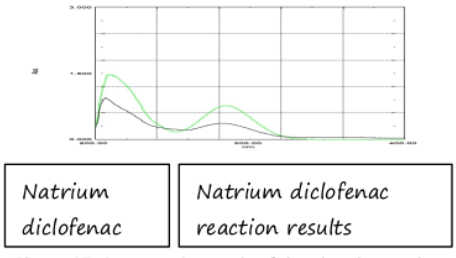


Figure 17. Comparative study of the absorbent value of diclofenac sodium

From the above data, it could be discussed, that there was a change in the spectroscopic character of diclofenac sample compounds, which were treated by reacting with yeasts. It was known that yeasts contained enzymes that had a chemically active side that was useful for catalyzing a reaction system. From the data it seemed that the diclofenac sodium character pattern underwent chemical changes related to changes in its structure, due to changes in its maximum wavelength pattern, and it might be calculated that there was a decrease in the number of diclofenac samples as follows:

Table 1. The biomodification ability of Saccharomyces yeasts to diclofenac test samples

Sample	Maximum Wavelength	Absorbency	Percentage in sample quantity
Natrium diclofenac	257nm	1500	50.67%
Sodium diclofenac results from a reaction with *Sacch. cerevisiae* yeasts	257nm	740	

From the data, it was known, that local Saccharomyces cerevisiae yeast had a good catalyst ability, proven to be able to reduce the sodium diclofenac sample that is significantly

over half in available quantity. This was a prospect for further research by utilizing local native Indonesian materials. Commercial yeasts were a blend of organic chemical ingredients that served as a filler for microbes. The microbes in these yeasts were a source of enzymes, in terms of being used as an enzyme for the hydrolysis of starch into simple sugars. But it was known that in Saccharomyces species, it contained other enzymes, so it was most possible that other organic ingredients besides starch could also undergo further reactions. In this study, it was proven that the sodium diclofenac model sample might experience biomodification. Further research might be able to deepen the qualitative changes and analysis of the products formed, as well as those related to aspects of their toxicity.

For ibuprofen samples reacted with yeasts, the measurement results using a UV-Vis spectrophotometer as shown in the following image. From each absorbency it had between ibuprofen and ibuprofen samples produced by yeasts seemed any difference, and in the comparative study of both, it was seen that there were no significant changes. When compared with the sodium diclofenac sample, the biomodification results were significantly different.

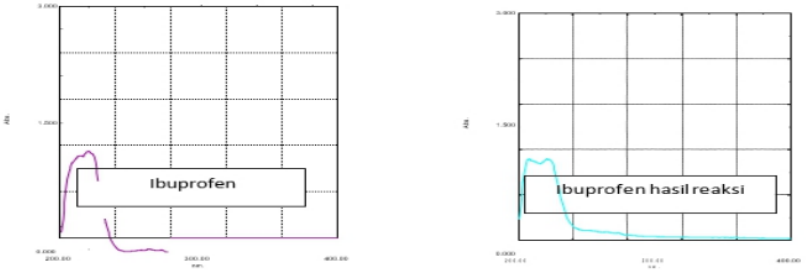


Figure 18. Comparative study of the absorbency value of ibuprofen

In quantitative calculations, the data resulted in a decrease in the quantity of ibuprofen by 19.25%. This change illustrated the initial principle, that ibuprofen could undergo enzymatic changes and decrease in number. It was known that biocatalyst enzymes had active reactive groups that might react with an organic compound.

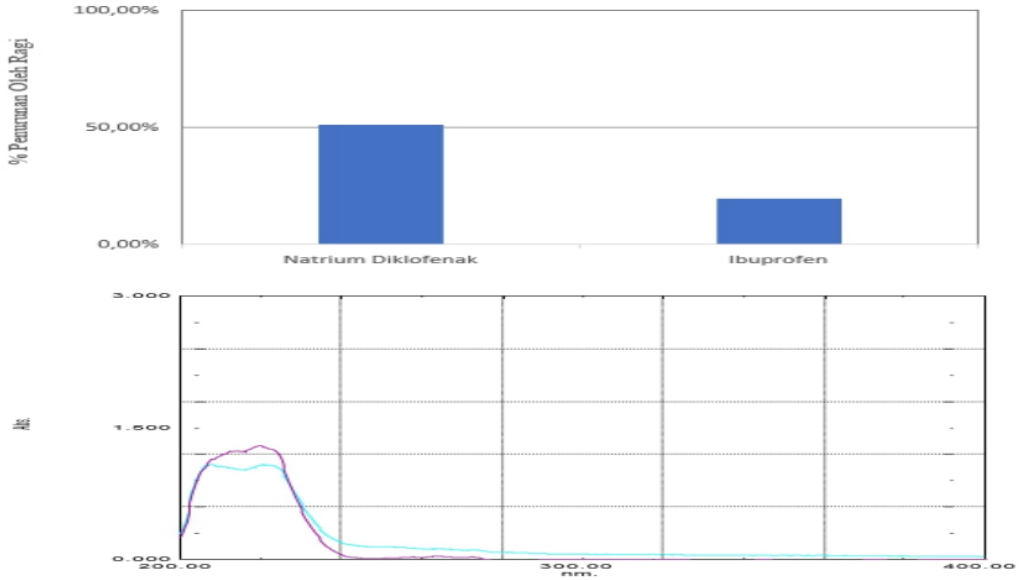


Table 2. The biomodification ability of Saccharomyces yeast to ibuprofen test samples

Sample	Maximum Wavelength	Absorbency	Percentage in sample quantity
Ibuprofen	257nm	1,300	19.25 %
Ibuprofen results from a reaction with *Sacch. Cerevisiae* yeast	257nm	1050	

It was known that the functional groups in ibuprofen, namely aromatic, carboxylic and amine, were expected to undergo structural changes, and from observations of research results, there was only a change of about 20%, then of the three functional groups, it was presumed that the functional groups that experienced the most changes were

the only carboxylate. When compared with the diclofenac sample, it was known that this sample had more than half of the modifications, so it could be presumed that the available function groups namely aromatics, halogens, and carboxylates were susceptible to change. From other studies, it was reported that the process of dehalogenase by enzymes was very possible because the carbon-halogen bond was polar so that it was vulnerable and easily broken.

CONCLUSION

1. Local commercial *Saccharomyces cerevisiae* yeasts have the capacity as an adsorbent material for model samples for pharmaceutical waste namely sodium diclofenac and ibuprofen, and good adsorbent properties and can be influenced by acid and base conditions.
2. The ability of Saccharomyces yeasts as an adsorbent follows the pattern of non-chemical physical adsorption mechanisms and can reduce by 20-30%.
3. Local commercial *Saccharomyces cerevisiae* yeasts can serve as a biomodification agent for samples of pharmaceutical waste, sodium diclofenac, and ibuprofen as indicated by the detection of other products in the form of nodes in the TLC test on sodium diclofenac, which means that the sample has undergone chemical structure changes. However, this ability does not occur in ibuprofen samples, this shows that sodium diclofenac is more easily degraded, and ibuprofen is more stable than sodium diclofenac.
4. Chemically, *Saccharomyces cerevisiae* yeasts through fermentation for a period of 3 x 24 hours can reduce the concentration of test samples by 50% for sodium diclofenac and 20% for ibuprofen.
5. Local commercial *Saccharomyces cerevisiae* yeasts can be used as an alternative material for pharmaceutical waste treatment with a physicochemical approach, with a simple method and with a relatively inexpensive material.

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