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Research Article

Rapid Purification of Glycerol by-product from Biodiesel Production through Combined Process of Microwave Assisted Acidification and Adsorption via Chitosan Immobilized with Yeast

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Abstract: Biodiesel is a proven alternative to the petroleum diesel fuel. During biodiesel production, glycerol is produced as a by-product. This by-product consist of impureties such as soap, salts, sodium catalyst and so on. Traditionally, two of the most conventional techniques that is applied to glycerol purification are distillation and ion-exchange. These techniques are, however, still expensive to generate pure glycerol. Recently, several alternative "combination" treatment procedures have been used. These treatment has several advantages over others methods such as producing large amounts of glycerol-rich layer that requires simple treatments and not causing any high operational cost. In this study, the combination treatment process have been used in order to reach high glycerol content. Basically, these stages starts with using microwave assisted acidification process and the next process utilizing a bioadsorbent synthesized from dead yeast cells immobilized on chitosan. The final yield of glycerol was about 93.1-94.2% (w/w).

Keywords: Bioadsorbent, chitosan, glycerol, inactive yeast, microwave

INTRODUCTION

The world today is facing the problem of increasing fossil fuel prices and, therefore, the development of future alternative fuels has become necessary and challenging. One of the feasible alternatives for alleviating the demand for fossil fuels, especially in the transportation and agricultural sectors, is the utilization of biomass to produce biodiesel (Wilson, 2002; Singhabhandhu and Tezuka, 2010). Biodiesel is renewable, biodegradable, non toxic and has almost very close property to that of fossil-based diesel and hence is one of the most promising alternative fuels. It can be produced form vegetable oil as well as animal fats. Biodiesel is commonly produced via tranesterification process and the triglycerides are converted into esters (biodiesel) by the transesterification reaction. (Fazal *et al*., 2011). The production of 10 kg of biodiesel by the transesterification process yields approximately 1 kg of crude glycerol as by-product (Kongjao *et al*., 2010; H'ajek and Skopal, 2010). Currently the world's capacity for biodiesel production is increasing dramatically and further increase in biodiesel production rates will significantly raise the quantity and surplus of crude glycerol and may have environmental effect.

Glycerol or 1, 2, 3-propanetriol is the principle by-product generated from transesterification of vegetables oils and animal fats. The crude glycerol discharged from biodiesel production plants consists not only of glycerol but also many other chemicals, such as water, organic and inorganic salts, soap and alcohol, traces of mono and di-glycerides and vegetable colors (H'ajek and Skopal, 2010). These impurities vary according to the type of oil and catalyst used in the biodiesel production (Pinto *et al*., 2011). High-purity glycerol is still required as it is an important industrial feedstock for applications in the food, cosmetic and pharmaceutical industries, as well as other more minor uses. A purified or refined glycerol is generally sold as 99.5-99.7% pure in the market. The common purified glycerol available in the market is manufactured to meet the requirements of the United States Pharmacopeia (USP) and the Food Chemicals Codex (FCC) (Pachauri and He, 2006). Pure glycerol can be utilized in the pharmaceutical and food industries and can also be easily oxidized, reduced, halogenated, etherified and esterified to obtain alternative commodity chemicals (Javani *et al*., 2012). However, crude glycerol originating from biodiesel industry is expensive to be purified to above 99% for use in food, pharmaceuticals, or cosmetics industries and so cheaper efficient refining methods are still required (Johnson and Taconi, 2007). In addition, waste glycerol from

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biodiesel production is classified as a waste under Schedule S181 of the Environmental Regulations in Malaysia and stored in drums and disposed off in landfills. The cost for landfill is about RM 500 (USD 166) and incineration cost RM 900 (USD 300) to RM 3000 (USD 1000) per ton (Hazimah *et al*., 2003). The processing technology of biodiesel production affects the characteristics of by-products and this is expected to influence the composition and utilization of crude glycerol. As biodiesel production has increased exponentially, the crude glycerol generated in this process has also been generated in a large quantity and is expected to grow steadily in the future. Furthermore, the economics of biodiesel might also be influenced by the way glycerol co-products are used (Suppes, 2006). Development of cheap and efficient processes to refine/purify crude glycerol is very important to the biodiesel production as it can improve the economic feasibility of the biodiesel industry. If crude waste glycerol could be purified cheaply, it can be used as feedstock for other oleochemical industries (Ayoub and Abdullah, 2012). Alternate ways of using the crude glycerol phase have recently been studied. Possibilities such as combustion, co-burning, composting, animal feed, thermochemical conversion and biological conversion have been applied to crude glycerol processing (Luo *et al*., 2008; Slinn *et al*., 2008; Valliyappan *et al*., 2008; H'ajek and Skopal, 2010). Efforts are also being made to convert of the surplus levels of glycerol and impure glycerol into bioethanol, the technique is neither widely available nor commercially viable at present.

Many conventional techniques for removal of impurities from crude glycerol have emerged such as distillation and ion-exchange. The distillation is the most commonly practiced method for purifying glycerol. The advantages of the distillation process are well known. However, the distillation of glycerin is an energy-intensive process. Glycerol has a high heat capacity, which demands a high-energy input for vaporization. Similarity ion-exchange techniques have also long been applied to glycerin purification. However, the high salt content of glycerin issued from biodiesel production makes ion-exchange uneconomical for this application. Specifically, the chemical regeneration cost for the resins becomes exceedingly high when salt contents approach the 5-7%; which is the percent-range commonly found in the biodiesel industry. Activated carbon has been the most popular and widely used adsorbent in wastewater treatment applications throughout the world. This is due to its high adsorption capacity, high surface area, microporous structure and high degree of surface reactivity (Babel and Kurniawan, 2003; Ozcan *et al*., 2004). In spite of its prolific use, activated carbon remains an expensive material since the higher the quality of activated carbon, the greater it costs. Therefore, it not attractive to be used in small-scale industries because of cost inefficiency. Hence, research interest into the production of alternative adsorbent to replace the costly activated carbon has intensified. In recent years, of the many sort of biosorbents recently investigated for the biosorption ability of heavy metals, *Saccharomyces cerevisiae* has proven to be the most promising as well as reliable and (Chen and Wang, 2008). Lately, the usage of dead bacterial cells for heavy metals removal has emerged as an alternative, since living cells have their own inherent limitation such as less efficiency and sensitive operating conditions (Chen and Wang, 2008; Farooq *et al*., 2010). It was found that various functional groups present on their cell wall offer certain forces of attractions for the metal ions and provide a high efficiency for their removal. Use of dead materials has several advantages because there is no need of growing, no growth media is required and these materials are available as wastes or by-products (Farooq *et al*., 2010). The free bacterial cells are generally very small particles, with low density, poor mechanical strength and little rigidity. These cells may pose several problems such as solid–liquid separation, possible biomass swelling and inability to regenerate/reuse. These problems can be avoided by the use of immobilized cell systems. The immobilization of the biomass in solid structures would create a biosorbent material with the right size, mechanical strength, rigidity, porosity and minimal clogging under continuous-flow conditions, which are important factors necessary for use in practical processes (Hasan and Srivastava, 2009; Zhou *et al*., 2009; Wang and Chen, 2006; Wang and Chen, 2009).

In this study, the uptake of impurities by some *Saccharomyces cerevisiae* as a biosorbent was carried out. The main objective of this study was to investigate the new processes for purification and recovery of glycerol through uptake of impurities from the crude glyscerol, a by-product of biodiesel production by non-living *Saccharomyces cerevisiae* biomass inmobilized on chitosan. The main focus will be to possibilly improve the efficiency of the impurities uptake from the crude glycerol by *S. cerevisiae* through pretreatment, immobilization and the use of microwave irradiation.

MATERIALS AND METHODS

Yeast strain and chemicals: Crude Glycerol (by product of Biodiesel process) was obtained from Uniten Biodiesel laboratory. *Saccharomyces Cerevisiae,* Chitosan (75% deacylated, medium molecular weight) and Glutaraldehyde were purchased from Merck, Germany. Bacto-Yeast Extract Peptone (YEP) was purchased from BD Diagnostic Systems USA. Sulphuric acid, charcoal powder, sodium hydroxide, potassium hydroxide; were all purchased from Sigma-Aldrich (USA). All the reagents and chemicals used were of analytical-reagent grade and used as received.

Cultivation medium: One hundred ml of YEP broth media (10 g/L yeast extract, 10 g/L Peptone and 5 g/L NaCl) was autoclaved in a 250 mL flask at 121°C for

15 min. After cooling, the dry *S. cerevisiae* (5 g) were aerobically propagated at 37°C in the YEP media while shaking it at 250 rpm using a mechanical shaker (Jeiotech Company, model SK-300). After 24 h the yeast cells were collected from the media by centrifugation at 10000 rpm for 5 min (Hettich Zentrifugen Rotofix 32). The prepared *S. cerevisiae* cells were then washed twice with distilled water. Ten $gram_{wet}$ cells were suspended in 200 mL of distilled water to mixed throughly to destroy aggregated cells and transfered to a beaker for ultrasonic treatment.

Ultrasonic pre-treatment: Ultrasonic treatment was applied to the rehydrated yeast cells at 30°C. In this setup, sample in batches of 200 mL each were sonicated using Cole-Parmer 130-Watt Ultrasonic processor (transducer: lead zirconate titanate piezoelectric). The frequency was 19 kHz and power input was adjusted between 0 to100 W. Different ultrasonic intensity (0.20, 0.30 and 0.50 W/mL) and sonication times (1, 5, 10 and 30 min) were applied. The transducer was mounted on a retort stand and the probe was lowered into the solution. The beaker containing the yeast was placed on a magnetic stirrer and later yeast cells were collected from the media by centrifugation.

Immobilization of dead yeast cells on chitosan beads: The yeast cells were immobilized on the chitosan beads. Firstly, chitosan solution was prepared by dissolving 3.0 g of chitosan powder in 50 mL of 3% (v/v) acetic acid solution. The chitosan solution was left overnight to ensure all of the chitosan flakes were dissolved. The chitosan solution was then added dropwise by using a model 100 push-pull syringe pump into a precipitation solution containing 450 mL of 0.5 M NaOH and 10 mL of 25% glutaraldehyde solution (as cross-linking agent). The entire mixture was mix gently (45 rpm) for 45 min. The crosslinked-chitosan beads were washed thoroughly 0.2 M Na-acetate buffer, pH 5.0. A solution of dead yeast cells (250 mg/g particles) was added and the mixture was stirred at 25°C for 25 min and then left at 4°C overnight. The next day the beads were washed until no cells were detected in the washing. The Chitosan beads immobilized with yeasts were stored at 4°C in 0.2 M Na-acetate buffer, pH 5.0 (Ivanova *et al*., 2011).

Crude glycerol refinement steps: Crude glycerol (by product of the biodiesel process) was obtained from Uniten biodiesel laboratory. A typical biodiesel production from the waste cooking oil normally produced about one litre of glycerol for every 5 litres of waste cooking oil transesterified (alkali-catalyzed transesterification). The crude glycerol was purified using a combination of the following treatments to obtained good quality purified glycerol, which could than be further used as a feed stock in other applications.

- **Initial purification of pretreated waste glycerol by reduction of free fatty acid and salt levels via microwave assisted acidification:** The glycerol obtained from the previous pre-treatment step was further treated to remove remaining free fatty acid and salt by acidification. Typically, about 500 g of the crude waste glycerol was transferred to a beaker and subjected to microwave irradiation using a microwave oven at frequency of 2.45 GHz at power output of 100 W for 3 min. After the irradiation, the solution was acidified by addition of H_2SO_4 (2 M) in small portions at a time to bring the solution to the desired pH (1, 3, 5 and 7). The mixtures were allowed to stand at room temperature until the solution had phase separated into two layers, the top layer of free fatty acids, the bottom aqueous layer of glycerol and inorganic salts. Finally, the upper free fatty acid phase was removed from the aqueous glycerol phase through a pipette.
- **Final purification of the partially purified glycerol using the bioadsorbent chitosan immobilized with yeast cells:** Finally, the rest of salts and any heavy impurities in the crude glycerol were removed by using the bioadsorbent prepared in this study; chitosan immobilized with yeast cells. Prior to bioadsorption of contaminants in the partially purified glycerol-rich layer, (obtained from purification step after acidification), the solution was neutralized by the addition of 5 M NaOH to pH 7.0 and left at room temperature for 5 min. Removal of impurities and colour was done in a batch system. The batch adsorption experiment was conducted in a 500 mL beaker and equilibrated using a magnetic stirrer. Two hundred millilitres of the partially purified glycerol (from purification step 1) was transferred to the beaker and then 5 g of the chitosan immobilized with yeast cells beads were added. The mixture was then subjected to short burst of microwave irradiation using a microwave oven at frequency of 2.45 GHz at power output of 200 W for 90 sec. After allowing it to cool for 3 min., the microwave irradiation was repeated two more times (total 3 times). At the end of the third microwave irradiation, the mixture was left at room temperature for 40 min with slow stirring at 150 rpm. At the end of 40 min, the beads were removed by decanting and the solution was analyzed for MONG, glycerol content, ash content and colour.

ANALYTICAL PROCEDURES

The recovered crude glycerol was characterized by the parameters below, obtained by the methods of analysis given: The water content was measured following the Standard method (ISO 2097-1972) by

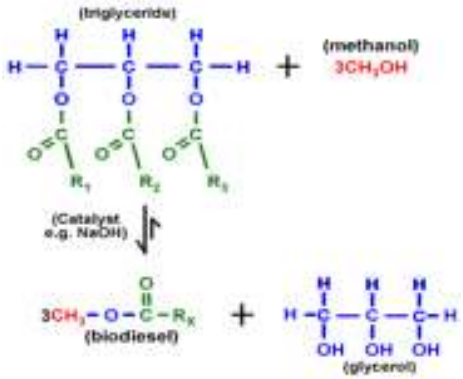

Fig. 1: Transesterification reaction of triglyceride (vegetable oil)

(a) (b)

Fig. 2: The colour of the as obtained crude glycerol from the biodiesel production unit (a) and the commercial pure glycerol (b)

using the Karl Fisher titration and was done using the Metrohm, 787 KF Titrino apparatus. Ash content was analyzed according to the Standard method (ISO 2098-1972) by burning 1 g glycerol in a muffle furnace at 750°C for 3 h (Fan *et al*., 2009; Kongjao *et al*., 2010). The matter organic non-glycerol (MONG) was calculated by the difference from a hundred of the previous three compositions [100-(% glycerol content +% water content +% ash content)] (Fan *et al*., 2009; Kongjao *et al*., 2010). The pH of the glycerol solution was measured with a pH meter (Mettler Toledo, MP220). The color of glycerol was measured by a UV/V is spectrophotometer (Jasco, V-530) at a wavelength of 457 nm. The standard test method for dynamic viscosity and density measurements were performed using Stabinger Viscometer according to ASTM D7042 method. This method specifies a procedure for the concurrent measurement of both the dynamic viscosity, η and the density, ρ. The kinematic viscosity, ν, can be obtained by dividing the dynamic viscosity, η, by the density, ρ, obtained at the same test temperature.

RESULTS AND DISCUSSION

Characteristics of the crude glycerol from a waste cooking oil via biodiesel production process: The recovered crude glycerol was a by-product from the transesterification process of waste cooking into biodiesel (methyl ester) obtained from the Uniten Biodiesel laboratory. The chemical reaction to obtain biodiesel and the waste glycerol is as shown in Fig. 1.

The as obtained crude glycerol was a dark brown thick liquid (Fig. 2a) with high alkaline pH (pH>9.6) compared with commercial glycerol (Fig. 2b). It was found to contain only between 36-37% (w/w) glycerol with a high content of ash, water and MONG contaminants and with lower density and viscosity compared with the commercial pure glycerol as highlighted in Table 1.

The Table 1 shows the comparison of characteristics of crude glycerol obtained from biodiesel by-product with the commercial grade glycerol (Sigma Aldrich). In most cases, waste glycerols from biodiesel production usually contain methanol, soap, catalysts, salts, non-glycerol organic matter and water impurities (Hansen, *et al*., 2009). The ash content from crude glycerol was high in the range of 3.3-4.1% (w/w), which was largely due to the catalyst salt especially NaOH or KOH originally used during the transesterification process. It has been reported that the salt content in waste glycerol from biodiesel production can be as high as 5-8% (wt/wt) and the residual methanol content as high as 28% (wt/wt) (Posada and Cardona, 2010). The water content was also high (12.3-14.5% w/w), which might be attributed to absorption of moisture from the surrounding during the transesterification (process. Glycerol is hygroscopic and can absorbs water from the surrounding. By far the largest contaminant

Table 1: Comparison of characteristics of crude glycerol obtained from biodiesel by-product with the commercial glycerol according to BS standards

Parameters	BS 2621:1979 (Ooi *et al*., 2001)	Crude glycerol from biodiesel process	Commercial glycerol (Sigma Aldrich)
pH	-	9.6-10.8	6.9-7.0
Glycerol content (wt.%)	>80	36.4-37.6	99.98
Ash content (wt.%)	<1.0	3.3 - 4.1	0.002
Water content (wt.%)	No	12.3-14.5	0.01
Matter organic non glycerol (MONG, wt.%)	<1.5	53.5-64.5	0.001
1,3-propandiol (wt.%)	<0.5	NA*	NA*
Density at 20°C (g/cm^3)		1.014	1.267
Kinematic Viscosity at 40°C (Centistokes; cSt)		267.7	46.8
Color types	-	Dark Brown	Clear

*: Not analyzed

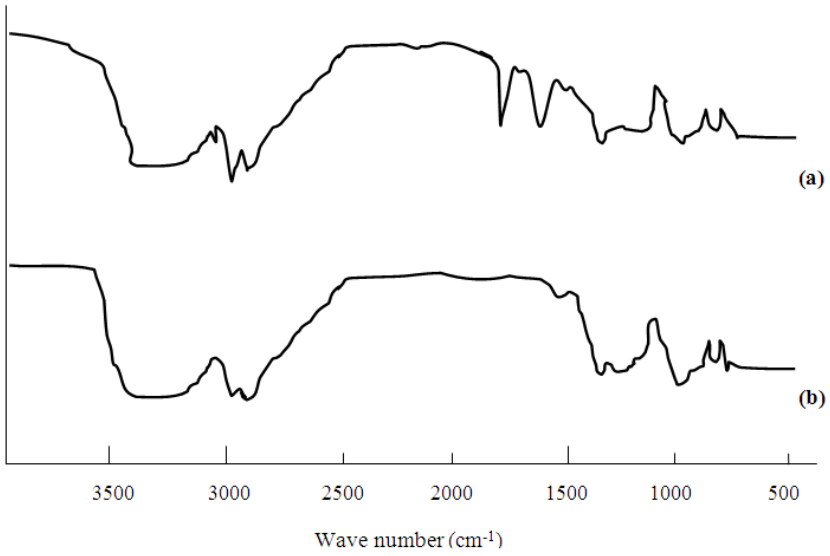

Fig. 3: Representative FTIR spectra of the crude glycerol (a) and a commercial glycerol solution (b)

was MONG (53.5-62.8% (w/w)), which actually surpassed the glycerol levels in the crude glycerol waste. The MONG was generated by the contamination of soap, methanol and methyl esters in the glycerol residue from the biodiesel production process (Kongjao *et al*., 2010). During the transesterification stage and phase separation process, some of the free fatty acids were released as soluble soap and some of methyl esters dissolved or suspended in the glycerol solution. These free fatty acids and methyl esters then reacted with the excess NaOH in the to re-form soap which remained in the glycerol residue (Johnson and Taconi, 2007). The impact these impurities have on the bioconversion process needs to be investigated. One or more of these substances could be a problem as they may inhibit growth of microbial cells. In addition, methanol is considered a hazardous waste and is non-biodegradable. So it is imperative that a methanol removal pretreatment system be employed to prevent crude glycerol from becoming an environmental threat (Rumbold *et al*., 2009).

The FTIR analyses were performed to investigate the changes in the functional groups. The composition of the crude glycerol (Fig. 3a) was analyzed by FTIR and compared to commercial glycerol (Fig. 3b). The main functional groups of commercial glycerol, including the O-H stretching at 3.300/cm, C-H stretching at 2.890 and 2.990 cm^{-1}, C-O-H bending at 1.370 to 1.430/cm, C-O stretching from 1100cm^{-1} as primary alcohol to 1450/cm representing secondary alcohol, O-H bending at 930/cm and also the H_2O bending at 1,620/cm were clearly resolved. The spectra of the waste crude glycerol obtained from the waste used-oil biodiesel plant (Fig. 3a) additionally showed strong FTIR peaks at 1.590/cm and 1.750/cm and a small band at 3.060/cm, indicating the presence of some impurities. The sharp band at 1.590/cm represented the presence of COO^- functionalities of soap. The sharp peak at 1.750/cm indicated the presence of C = O compound (s) of an ester or carboxylic acid of fatty acid. The small band appearing at 3.050/cm indicated the presence of unsaturated C = C compound (s).

Initial purification of waste glycerol by microwave assisted acidification: To attain better purity of the glycerol and to remove impurities and other extraneous matter, the crude glycerol sample was firstly subjected to microwave assisted acidification process. In this study to obtain higher purity glycerol rapidly, the waste glycerol solution was initially subjected to preheating with microwave irradiation so as to achieve a lower activation energy which in turn will increase the rate of reaction. Improved rates of reaction of chemical reaction have been observed under microwave irradiation. It was reported that such irradiation is effective for not only heating during the reaction but also preheating (Asakuma *et al*., 2011). In their study on biodiesel production, Asakuma *et al*. (2011), have confirmed that preheating samples with microwave irradiation improves reactivity and the reason was due to the resultant lower dipole moment and lower activation energy (Asakuma *et al*., 2011). The acidification was than performed by addition of H_2SO_4 (2 M) in small portions at a time to bring the solution to the desired pH (1, 3, 5 and 7). Upon acidification the waste glycerol solution separated into two distinct layers, the glycerol and inorganic salt layer on the bottom and the free fatty acid layer on the top. Kongjao *et al*. (2010), had reported that after the addition of H_2SO_4 the crude glycerol can be automatically phase separated into three distinct layers comprised of a free fatty acid layer on the top, a glycerol-rich layer in the

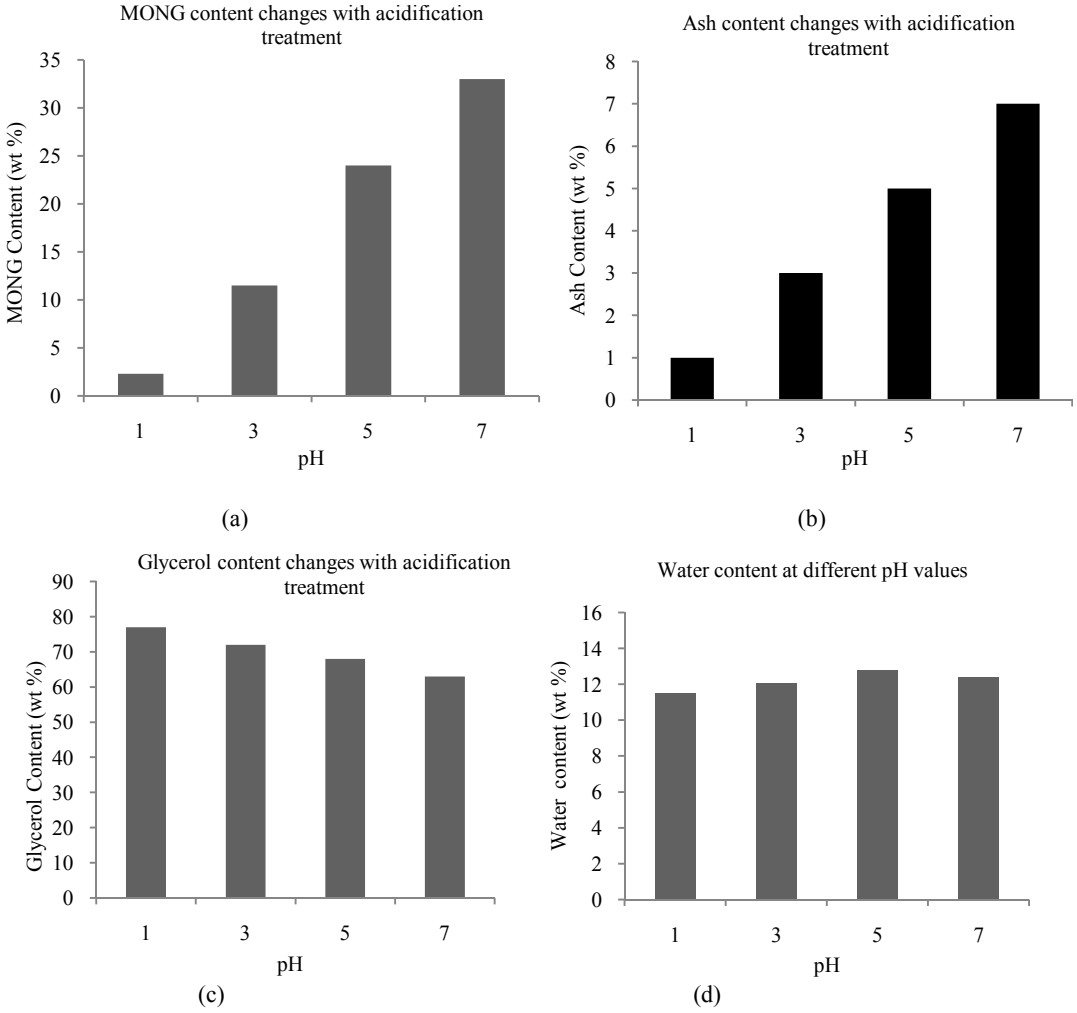

Fig. 4: Assessment of the (a) MONG, (b) ash, (c) glycerol and (d) water contents after acidification of the glycerol-rich layer with H_2SO_4 at the indicate pH

middle and, a small fraction of an inorganic salt layer on the bottom. In this study, however, the separation into two phases (fatty acid and glycerol-rich layers) was clearly observed after the acidification by H_2SO_4 for all investigated pH values. Similar results were reported by Hazimah *et al*. (2003) and Manosak *et al*. (2011).

The mineral acid will produce H^+ which will react with the soap bulk to convert it into insoluble protonated free fatty acids, which can float out as a top layer. The excess SO_4^{2-} from the ionized H_2SO_4 can couple with the sodium ions from the contaminated salts in the crude glycerol to form the relatively insoluble Na_2SO_4 in aqueous solution, which precipitates out as the bottom layer. With respect to acidification by H_2SO_4, the formation of inorganic salt layer in the acidified crude glycerol was not observed, which is contradictory with the results of Kongjao *et al*. (2010). This is likely to be caused by the presence of a high water content in the crude glycerol utilized in our study, which at ~14% (w/w) was approximately 3-fold higher than that in the study of Kongjao *et al*. (2010). With high water content, a large amount of acidic salt ($NaHSO_4$) can dissolve in the water resulting in the absence of an inorganic salt layer in crude glycerol.

Indeed, the pH of the acidification stage during the chemical treatment had a significant effect on the composition of the glycerol-rich layer. The composition of the glycerol-rich layer obtained was analyzed for glycerol, ash, MONG and water contents as shown in Fig. 4. The ash contents in the purified crude glycerol were lower than the original level in waste crude glycerol. It was observed that at low pH the content of ash was even lower than when treated at a higher pH. As shown in Fig. 4, the ash level dropped from about 2.1% at pH 7 to around 0.5% (w/w) at pH 1. This might be due to the excess sulphate ions from the ionized H_2SO_4, added during the acidification stages, complexing with the sodium ions from the contaminated salts in the crude glycerol to form the relatively insoluble Na_2SO_4 in aqueous solution,

particularly at a low pH (Ooi *et al*., 2001), hence the reason for low salt in the crude glycerol layer purified at a low pH, measured in terms of ash content.

In the case of MONG, its fraction in the glycerol-rich layer, at all pH values used, was lower than that in the crude glycerol before acidification treatment. Large amount of free fatty acid was separated from the crude glycerol under strong acid conditions, leading to a lower amount of MONG in the glycerol-rich layer. As seen in Fig. 4, the MONG level dropped from about 33% at pH 7 to around 2.3% (w/w) at pH 1. Similar results, but with slightly higher value of MONG was also observed by Kongjao *et al*. (2010). This shows that it is difficult to completely eliminate MONG contamination in the glycerol by the acidification method. The reason for this as mentioned previously is the re-formation and contamination of the glycerol by soap formed from the reaction between excess NaOH and the dissolved short (C6-8) and medium chain (C10-14) fatty acids, generated from the soap hydrolysis in the acidic stages and then dissolved in the polar glycerol phase rather than partitioned into the upper free fatty acid phase (Wang *et al*., 2008). The water content changed slightly, within the range of 11.5-12.8% (w/w), during the acidification process. The glycerol content, increased with the lowering of the pH during the acidification process, since at a low pH large quantities of fatty acid and salt were eliminated resulting in a concomitant increase in the relative glycerol content. The content increased to about 63-77% (w/w) from the value of about 36.4-37.6% (w/w) in the waste crude glycerol. The colour of the glycerol solution was reduced drastically after this purification stage, but was still not clear, having a rather light brown tint. This might be attributed to the presence of some minor amounts of saturated fatty acids, as well as other unknown compounds.

Final purification of the partially purified glycerol using the bioadsorbent chitosan immobilized with yeast cells: In the present study, the non-living cells of *S. Cerevisiae* immobilized on cross-linked chitosan used as the biosorbents plus microwave irradiation were utilized for efficient adsorption of metal ions, colour, MONG, ash and other pollutants from partially purified waste glycerol solutions. *Saccharomycess cereviceae* was applied as adsorbent because it has very high surface area that is suitable for absorption. A number of processes (physical and chemical adsorption, electrostatic interaction, ion exchange, complexation, chelation and microprecipitation) take place essentially on the polysaccharide coating of most bacterial cell wall (Aksu, 2005). The carboxyl and phosphate groups of compounds like the peptidoglycan, teichoic acids, teichuronic acids and lipoteichoic acid which constitute the cell wall of the Gram-positive bacteria cells, give an anionic character. This anionic cell wall attacks cationic ions and sorption of metal ions takes place on surface of cell wall (Rai *et al*., 1998). Presence of phospholipids, lipopolysaccharide and peptidoglycan of Gram-negative bacteria is chiefly responsible for its metal binding ability (Rai *et al*., 1998). Dead cells have been shown to accumulate pollutants to the same or greater extent than growing or resting cells (Aksu and Dönmez, 2003).

Microbial biosorbents are basically small particles, with low density, poor mechanical strength and little rigidity. The most important problem includes the possible biomass swelling, inability to regenerate/reuse and development of high pressure drop in the column mode (Vijayaraghavan and Yun, 2008). Through immobilization of biomass, the mechanical and chemical strength as well as porosity will be higher. Immobilization of microorganisms within a polymeric matrix (such as chitosan) exhibited greater potential, with benefits including the control of particle size, regeneration and reuse of the biomass, easy separation of biomass and effluent, high biomass loading and minimal clogging under continuous-flow conditions (Hasan and Srivastava, 2009; Zhou *et al*., 2009). The chitosan were crosslinked with glutaraldehyde to improve the wet strength of the chitosan beads (Wan Ngah *et al*., 2002). As reported by Shu and Zhu (2001), after the modification the chitosan beads had improve mechanical strength, chemical stability, hydrophobicity and also biocompatibility. The chitosan not only serves as a support matrix but is also a very good adsorbent. Hence the adsorption potential of the bioadsorbent produced by the immobilizing of *Saccharomyces cerevisiae* on to chitosan was highly improved.

Using microwave irradiation, it is often possible to accelerate the rate of reactions and hence reduce the reaction time and the energy consumption (Saifuddin and Chua, 2004; Hernando *et al*., 2007). It is anticipated that at low power level of microwave irradiation non-thermal effects of microwave play a role. At low power level the bonds of the reactant molecules may undergo conformational changes to favor the cleavage of the bonds, which enhances the rate of reaction. Non-thermal effects or microwave effect has been observed in a number of microwave assisted catalytic or enzymatic reactions (Yadav and Lathi, 2007; Saifuddin *et al*., 2011). In their study on biodiesel production, Asakuma *et al*. (2011), have confirmed that preheating samples with microwave irradiation give the molecules a more flattened configuration, which improves reactivity due to the resultant lower dipole moment and lower activation energy. A more flattened and exposed molecular structures will have greater surface area, which enhances their polymer adsorption onto the adsorbent (Azcan and Danisman, 2008). In the present study, it is envisaged that microwave treatment exposes certain pertinent bonds and hence allows greater binding of molecules, ions and other similarly charged particles

Table 2: Comparison of characteristics of the final purified glycerol with the crude waste glycerol and other treated glycerol solution from acidification treatment

Parameters	BS 2621:1979 (Ooi *et al*., 2001)	Commercial glycerol (Sigma Aldrich)	Crude glycerol from biodiesel process	Partially purified glycerol after acidification treatment (pH 1)	Final purified glycerol
pH	-	6.9-7.0	9.8-10.8	-	7.2-7.4
Glycerol content (wt. %)	>80	99.98	36.3-37.6	77	93.1-94.2
Ash content (wt. %)	<1.0	0.002	3.3-4.1	0.5	0.008-0.01
Water content (wt. %)	No	0.01	12.1-14.2	11.5 - 12.8	8.9-10.5
Matter organic non glycerol (MONG), (wt. %)	<1.5	0.001	53.5-64.5	2.3	0.4-1.2
Colour types	-	Clear	Dark brown	Pale light brown	Clear

Table 3: Comparison of purified crude glycerol properties obtained from this work with other works

Author (s)	Source of crude Glycerol	Kind of Treatment	Glycerol (wt. %) (i)	Glycerol (wt. %) (ii)	Ash (wt. %) (i)	Ash (wt. %) (ii)	MONG (wt. %) (i)	MONG (wt. %) (ii)	Water (wt. %) (i)	Water (wt. %) (ii)	Colour
Asher and Simpson (1956)	Soap lye solution	Ion extraction	7.5	82.5	13	7	-	-	-	-	-
Ooi e*t al*. (2001)	Transesterification of palm kernel oil	Chemical and physical treatment	17.7	51.4	58.7	13.8	17.7	25.9	5.9	8.9	-
Hazimah *et al*. (2003)	Fatty acid plant	Chemical and Vacuum Distillation	70	99.3	4	7	-	-	-	-	-
Hájek and Skopal (2010)	Saponification of oil during biodiesel production	Methanolysis treatment	51.3-58.9	86	-	-	-	-	-	-	-
Manosak *et al*. (2011)	Transesterification of waste used-oil	Chemical and Physical treatment	36.7± 0.49	96.2± 0.03	4.31± 0.027	2.08± 0.06	44.0± 0.44	1.50±0.07	14.7± 0.90	0.06± 0.02	Clear
This work	Transesterification waste cooking oil	Chemical and Bioadsortion treatment	36.3-37.6	93.1-94.2	3.3 - 4.1	0.008-0.01	53.5-64.5	0.4-1.2	12.1-14.2	8.9 - 10.5	Clear

i: Concentration of glycerol and impurities in the original waste crude glycerol before treatment; ii: Concentration of glycerol and impurities in the purified glycerol after treatment

onto the bacterial cell wall components. The synergistic effect of cationic character of the chitosan amino groups, *S. cerevisiae* and the microwave irradiation brings about enhanced performance for effective adsorption.

At the chosen dose of chitosan immobilized yeast cell bioadsorbent a higher colour reduction was achieved, with a clear colour solution being obtained. As for the ash content the bioadsorbent was able to reduce it to less than 0.01% (w/w) which was well within the exceptable value (Table 2). The water and MONG and glycerol content proportions were slightly changed after the adsorption with the bioadsorbent. Water and MONG content was decrease to 8.9-9.8% and 0.4-1.2% (w/w), respectively. The glycerol content was higher, with about 93-94% after the bioadsorbent treatment. The pH of the purified glycerol was about 7.2-7.4 (Table 2). Some of the fatty acids, such as lauric acid and myristic acid, as well as the oxidation products of glycerol, e.g., dihydroxyacetone, glyceraldehyde, hydroxyl pyruvic aldehyde and tatronic dialdehyde and some unknown compounds were eliminated during this stage. The characteristics of the refined crude glycerol after the final adsorption stage were all in the acceptable range of values of the BS 2621:1979 (Table 2) except for water content which was still slightly higher than the value based on BS 2621:1979.

The summary of the properties of the purified crude glycerol of this study compared with the other works in shown in Table 3. It is obvious that the present treatment method is remarkably effective, cheap and rapid in the purification of the waste crude glycerol obtained from the by-product of the biodiesel production. Also noteworthy is that the percent of purified crude glycerol obtained from this study was higher than that from the work of Asher and Simpson (1956) and Ooi *et al*. (2001) and Hájek and Skopal (2010). This might be attributed to the utilization of much effective bioadsorbent during the adsorption step. With respect to the effect of purification procedure, it seems to be that our treatment was more effective and superior as compared to the rest although the chemical and vacuum distillation (Hazimah *et al*., 2003) and Chemical and Physical treatment (Manosak *et al*., 2011) were marginally better in terms of percentage yield of glycerol. Therefore, our process is a simple, low cost, rapid process which provides a better yield of glycerol within the acceptable range of values of the BS 2621:1979.

CONCLUSION

Although crude glycerol generated from biodiesel production is waste product, it is rich in glycerol and diglycerol and therefore a worthwhile source of feedstock for useful chemicals. The original high content of ash, water, colour and especially MONG

was successfully removed by a combination of chemical and bioadsorption method. The characteristics of the refined crude glycerol after the final adsorption stage were all in the acceptable range of values of the BS 2621:1979 (Table 2) except for water content which was still slightly higher than the value based on BS 2621:1979. Finally, almost complete color (and some other components) reduction was obtained by using this treatment process. The percent of purified crude glycerol obtained from this study was 93.1-94.2% (w/w). Bioadsorbents were characteristic of broad sources, low-cost and rapid adsorption. This type of adsorbent is stable and could possibly be used for adsorption of other toxic metals in waste water systems. *Saccharomycess cereviceae* immobilized on chitosan has very good potential for other waste treatment systems. Brewer's yeast cells and chitosan represent an inexpensive, readily available source of biomass that has a significant potential for synthesis of the bioadsorbent. It is easy to prepare, eco-friendly and has good adsorption capacity. It is of agricultural and fishing industry wastes. The passive uptake of pollutants from aqueous solutions by using a combination of dead bacterial cell biomass immobilized on chitosan and microwave irradiation is novel, rapid and low cost and may be applied to other aqueous solutions contaminated with organic and metal ions.

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