Biological Refining: Novel Technique to Reduce the Rancidity of used Edible Oil

Charmi Dedhia, JayaPrada Rao Chunduri
Department Of Biotechnology, Mithibai College, Vile Parle (w), Mumbai

Abstract: *Used cooking oil (UCO) is considered as household hazardous waste and the disposal is a matter of environmental concern. In the developed countries such as USA, UCO is collected and converted as biofuel called VIESEL using enzymatic reaction. The recycling of used cooking oil follows a chain of distribution from five stars to roadside food stalls in large scale being a common phenomenon in developing countries like India and Malaysia. In the household conditions the heating of oils >375°C could lead to certain hazardous conditions and the reuse of oil may result in dangerous consequences. Hence, rancidity is considered one of the major quality control for reusing the oil. An attempt to improvise the used cooking oil quality back to its original stage has been made during the studies. The immobilized bacterial consortia of Bacillus spp I and II, Pseudomonas aeruginosa, Staphylococcus aureus, Aeromonas spp and Micrococcus spp have shown remarkable results in reducing the rancid conditions. Further studies are considered for possible large scale applications and their effectiveness of this novel method.*

Keywords*: Biological refining, improving oil quality, used cooking oil, Rancidity, Microbial consortia, Immobilization of microbes, Bacillus spp, Pseudomonas aeruginosa, Staphylococcus aureus, Aeromonas spp, Micrococcus spp*

I. INTRODUCTION

The oldest and common method of food preparation is deep frying. When oils are reused for cooking purpose or frying, they undergo physical changes in viscosity, colour and fatty acid composition. They also undergo chemical reactions such as oxidation, hydrolysis, and polymerization resulting in the formation of hydroperoxides and aldehydes [1] and chemical decomposition [2] leading to quick rancidity. The heat-induced process transforms healthy oils into dangerous oils regardless of the original nutrient content. Smoke point is the temperature at which the nutritional content of oil begins to rapidly degrade if heated beyond it. Smoke point of oils varies with type of oil and decreases at every time the oil is reused [3]. Reusing UCO can cause negative impact on the health hence often eating at outside or roadside eateries is discouraged and also their use as biodiesel could be an environmental pollutant in the long run.

Vegetable oils like Saffllower, canola, olive, groundnut in the presence of heat, light and oxygen; turn rancid in a short time. Studies indicated that rancid oils could be involved in tumour promotion and in initiation of tumour [4] also chemicals such as peroxides and aldehydes can damage cells and contribute to atherosclerosis [5].

Marine bacteria are widely used for microbial enhanced oil recovery (MEOR) [6], Crude Oil degradation [7] and Biosurfactant production [8]. Marine microbes from shore are also capable of degrading crude oil, bunker oil and vegetable oil along with the production of biosurfactants [9]. Current study is aimed at assessing the ability and efficiency of those marine microbes to reduce the rancidity of used cooking oils in edible range under laboratory conditions.

II. MATERIALS AND METHODS

Marine bacteria:

Five oil degrading bacteria isolated from marine coastal region of Shivaji Park, Mumbai such as *P. aeruginosa*, *S. aureus*, *Bacillus* spp I, *Aeromonas spp*, *Bacillus* spp II *and Micrococcus spp* were considered for the study [9]. Enrichment of culture was carried out in three consecutive batches each having a span of 3 days by using previous growth as inoculums for the next.

Substrate Sample:

Repeatedly used Groundnut oil & Safflower oils for deep fry cooking conditions (five times) were used as substrates for study. The oils were cooled to room temperature and were further used to study the efficacy of selected consortia for improving the rancid conditions.

DCPIP method for assessing the potential of consortia:

Efficacy of oil degrading bacteria was assessed using DCPIP technique. The mixture of 0.1 ml of microbial culture, 3 ml BH medium containing oil sample and 0.3ml of DCPIP was incubated at 37^{o}C. Bacteria capable of altering the oil components produce electrons which can take part in oxidation and reduction reactions. The oxidized (blue) and reduced (colourless) conditions of DCPIP will help in identifying the activity of oil degrading microbes [10]. Change in colour was observed at 660 nm against uninoculated blank tube periodically to assess the potential of consortia.

Immobilization of bacterial cells:

Isolates were immobilised to form double layer immobilized beads [11] using 8% St. Sodium alginate and 6% calcium chloride. The cell suspension with a minimum of $1.5x10^9$ CFU/ml (Mc Farland tube no.5) was added to the Sodium alginate in a ratio of 1:2 and the beads were prepared in chilled Calcium chloride. The resulting beads were hardened in calcium chloride for 1 hr, washed, wet sieved and surface dried in a laminar flow hood. When sufficiently dried, the beads were placed in a cell-free 4% (w/v) stirred sodium alginate solution for a further 30 min, to produce a second layer, and hardened in calcium chloride to prevent cell leakage from the beads [12]. The average diameter of the bead was 2-3mm and kept in the oil growth medium for activation prior to use for activation. Around 20 beads were added to 10 ml test sample (used cooking oil) to analyse their efficiency. Test oil sample along with beads were incubated at 37^0 C. Oil was checked at intervals of 24hrs to assess the improvement in the quality using different parameters.

Oil quality assessment:

Physical parameter:
Colour, Odour and Viscosity:

Colour, Odour and viscosity are the three physical parameters which indicated the quality of oil and may vary with the type of oil. Dark colour, Odour and highly viscous oils characterised these rancid oils after heating. However, the treatment resulted in change in colour, odour and viscosity was observed which was similar to that in pure conditions visually

Chemical parameters:

Peroxide value (PV):

Peroxide value is considered one of the important oil quality parameters. 1.00 (±0.05) g of sample into a 250 ml glass stoppered Erlenmeyer flask containing 30 ml of the acetic acid - chloroform solution followed by swirling the flask until the sample is completely dissolved by warming it on a hot plate carefully. The mixture was titrated against 0.1N Sodium thiosulphate solution using starch as indicator as suggested by the standard methods (AOAC, standard 965.33, 1997). A blank titration was performed with 30 ml the acetic acid - chloroform solution at each time.
S = titration of sample, B= titration of blank
Peroxide value = (S - B) X Normality of thiosulfate X 1000 / weight of sample (g)

Free Fatty acid analysis (FFA):

Free Fatty Acids (FFA) were determined by the method of Hoffpauir et al. [13] with modification. Samples were tested at 2-week intervals. FFA was determined by dispersing the lipid residue in a solution consisting of 25 ml of M-Cresol purple and 10 ml of Petroleum ether. The amount of 0.1 N alcoholic NaOH to change the yellow colour of the solution to greyish purple was recorded. A blank titration was performed with 25 ml of m-cresol purple and 10 ml of petroleum ether at each time. FFA per cent was calculated as oleic acid and expressed as a percentage of the total lipids.
FFA% = (ml titrated - mL blank) × 28.2 × concentration of alcoholic NaOH / weight of sample (g)

Statistical analysis:

Pearson correlation coefficient of the Peroxide value and free fatty acids was calculated using Microsoft excel software to understand interrelationship between these two parameters before and after treatment with selected consortia.

III. RESULTS

5 times used oil (Groundnut oil & Safflower oil) after cooling in room temperature were used for the assaying efficiency of oil degrading bacterial species based on the DCPIP reduction levels. The absorbance values of mixtures were taken periodically at 660nm to assess the decreasing trend of DCPIP's blue colour. The time for discolouration of DCPIP was considered as criteria to assess the oil degrading potential of isolates. Decolouration time of DCPIP is directly proportional to the hydrocarbons utilization by the individual isolate/consortium [10]. Isolates which decolorized DCPIP in the relatively shortest time <7 days were chosen for preparing a consortium. Based on DCPIP decolouration time 6 most efficient isolates were: *Bacillus spp I, Bacillus spp II, S. aureus, P. aeruginosa, Aeromonas spp, and Micrococcus spp*. *Bacillus spp I* and *S. aureus* had taken less time of 6 days in case of groundnut oil and *P. aeruginosa* took less than 4 days in case of Safflower oil to decolourize the DCPIP (originally blue in colour) in comparison with other isolates (Fig. I, II).

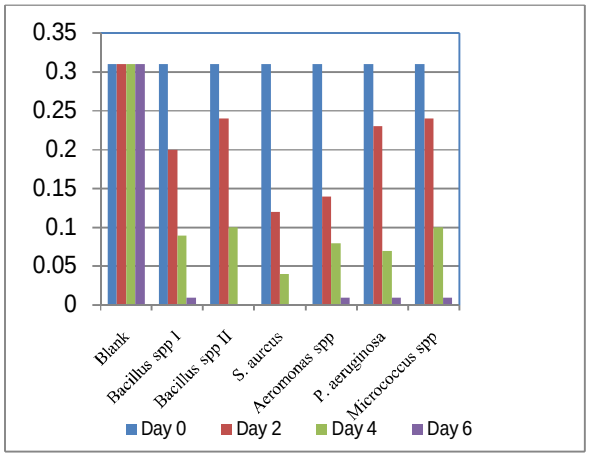

Fig. I. Decolouration of DCPIP with 1% Used groundnut oil by selected isolates

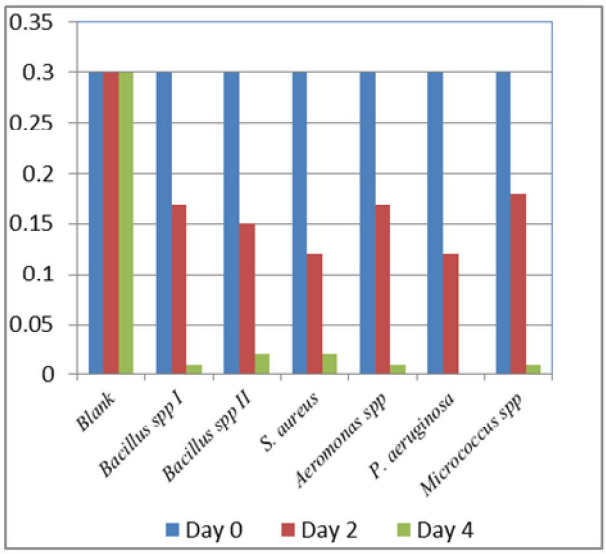

Fig. II: Decolouration of DCPIP with 1% Used safflower oil by selected bacterial isolates

These isolates were considered for double layer immobilisation in alginate substrate. The immobilised beads (around 20) were added to the UCO substrates and incubated at 37° C. Periodic investigations indicated changes in the texture, odour and colour. Peroxide value and FFA value were considerably improved after 2 weeks of incubation.

Peroxide Values (mEq/Kg) of used oil:

The peroxide value is the classical method for determining the extent of oxidative rancidity and measures the formation of intermediate peroxides in milliequivalents of active oxygen per kilogram of sample. Peroxides formed by fat oxidation react with iodide ions to form iodine, which in turn is measured by titration with thiosulphate.

During the current study, the peroxide values of the unused oils found to be low viz. Groundnut Oil: 1.1 ± 0.05 mEq/Kg and Safflower Oil: 0.7 ± 0.05 mEq/Kg prior to the use may due to the pure conditions. However, great rise in PV values was observed on multiply used ground oil (1.1 ± 0.05 to 10 ± 0.05) and unused Safflower Oil (from 0.7 ± 0.05 to 17 ± 0.42) from that of nascent ones. Maximum limit of peroxide value suggested by Alimentarius, C. [14] *is* 15 mEQ/Kg.

Peroxide values play an important role while assessing the rancidity level of oils and the level of use. The present treatment with bacterial consortia indicated reduction in peroxide values of used groundnut oil and used safflower oil from 10 ± 0.05 and 17 ± 0.42 to 1.8 ± 0.15 and 3 ± 0.01 respectively indicating the isolates capability in improving the quality. *Bacillus* spp *I* (from 10 ± 0.05 to 12 ± 0.04 for groundnut oil, from 17 ± 0.42 to 19.2 ± 0.29 for safflower oil) and *Micrococcus* spp (from 10 ± 0.05 to 10.7 ± 0.14 for groundnut oil, from 17 ± 0.42 to 18.1 ± 0.48 for safflower oil) increased the PV values on the contrary to the other consortia. S. aureus found to be most efficient in improving the PV value in both the cases after an incubation of two weeks (Table I).

Isolates/ Type of UCO	***Used groundnut oil***	***Used Safflower Oil***
Blank(Beforetreatment)	10 ± 0.05	17 ± 0.42
Bacillus spp I	12 ± 0.04	19.2 ± 0.29
Bacillus spp II	2.5 ± 0.06	5 ± 0.05
S. aureus	1.8 ± 0.15	3 ± 0.01
Aeromonas spp	2.8 ± 0.43	4.1 ± 0.16
P. aeruginosa	2.3 ± 0.24	4 ± 0.33
Micrococcus spp	10.7 ± 0.14	18.1 ± 0.48

Table I : Peroxide Value of Used cooking oil before and after treatment of incubation for 2 weeks (mEQ/Kg ± SD) with immobilized bacterial isolates

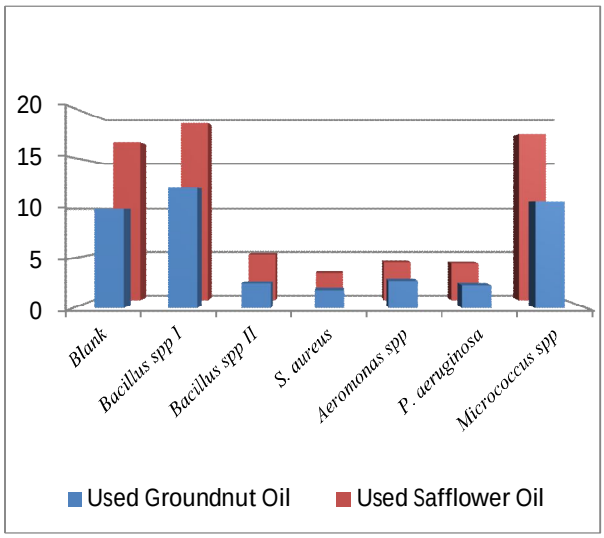

Fig III: Peroxide Value of Used cooking oil before and after treatment (mEQ/Kg)

Free Fatty Acid Value (%) for Used Cooking Oil:

FFA per cent was calculated as oleic acid and expressed as a percentage of the total lipids. The free fatty acid values of the unused oils was assessed prior to the study and found to be groundnut oil: 3.2 ± 0.05 % and Safflower Oil: 2.5 ± 0.05 %.

Great rise in FFA values on multiple use of unused oils was observed during the study viz., ground oil (from 3.2 ± 0.05 % to 7 ± 0.55 %) and Safflower Oil (from 2.5 ± 0.05 % to 10 ± 0.60 %) from that of the fresh ones. A fall in FFA content was however observed after inoculation of isolate's beads, bringing the values of used groundnut oil from 7 ± 0.55 % to up to 2 ± 0.15 % and, from 10 ± 0.60 % to up to 3 ± 0.26 % for used safflower oil within a period of two weeks incubation at 37^0C.

While *Bacillus spp I* (from 7 ± 0.55 % to 7 ± 0.78 % w.r.t. groundnut oil, from 10 ± 0.60 to 10 ± 0.89 w.r.t. Safflower oil) showed negligible effect, *Micrococcus spp* (from 7 ± 0.55 % to 10 ± 0.4 % w.r.t. groundnut oil, from 10 ± 0.60 % to 11.1 ± 0.48 % w.r.t. Safflower oil) increased the FFA values. *P. aeruginosa and Bacillus spp II* showed moderate effect. Whereas *Aeromonas spp (*from 7 ± 0.55 % to 2 ± 0.64 % w.r.t. groundnut oil, from 10 ± 0.60 % to 3 ± 0.75 % w.r.t. Safflower oil) showed almost equal efficiency as that of *S. aureus* (from 7 ± 0.55 % to 2 ± 0. 15 % w.r.t. groundnut oil, from 10 ± 0.60 % to 3 ± 0.26 % w.r.t. Safflower oil) which had better capability and efficiency for reducing FFA value.

Isolates/Type of UCO	***Used groundnut oil***	***Used Safflower Oil***
Blank	7 ± 0.55	10 ± 0.60
Bacillus spp I	7 ± 0.78	10 ± 0.89
Bacillus spp II	3 ± 0.20	4 ± 0.64
S. aureus	2 ± 0.15	3 ± 0.26
Aeromonas spp	2 ± 0.64	3 ± 0.75
P. aeruginosa	3 ± 0.85	4 ± 0.85
Micrococcus spp	10 ± 0.4	11.1 ± 0.48

Table II: FFA of Used cooking oils before and after treatment (% ± SD)

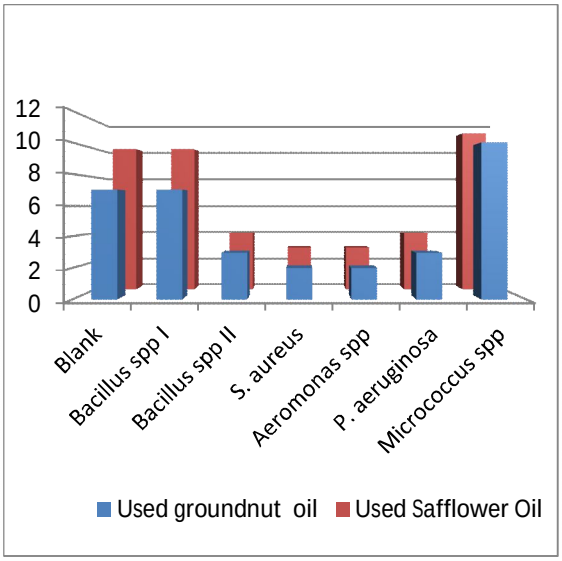

Fig IV: FFA of Used cooking oil before and after treatment %

A significant positive correlation was observed between Peroxide Value and Free Fatty acid value was observed (r = 0.98) for P < 0.05. The reduced values of peroxide indicate improved conditions (FAO). Similarly, the low FFA indicates the reduced rancidity of used cooking oil.

IV. DISCUSSION

Marine microorganisms in oil slick and oil degradation procedures have gained more prominence and are repeatedly used in the environmental restoration processes [15]. Different bacterial species capable of oil degradation are isolated from oil polluted sources like marine shores [9] have been studied. Current study aims at understanding the applicability of these marine microorganisms in reducing rancidity of used cooking oil.

Screening of bacterial isolates with high efficiency to degrade oils becomes necessary to initiate the studies of improving oil quality. By incorporating an electron acceptor such as DCPIP to the culture medium, it is possible to ascertain the ability of the microorganism to utilize the substrate by observing the colour change of DCPIP from blue (oxidized) to colourless (reduced) in a screening [16]. The principle of this technique is that, during the microbial oxidation of the carbon source, electrons are transferred to electron acceptors such as O_2, nitrates and sulphate. DCPIP was widely used as an electron receptor to observe oxidation and reduction reactions [10], as sensitive indicator to study biodegradation of biodiesel and vegetable oil [17]. Thus, the use of DCPIP redox indicator presents a rapid, simple and low cost tool for evaluating capability of different microorganisms to degrade different oils [18].

Role of Immobilized microorganisms as whole cells or enzymes for production of antibiotics [19], L-Lactate [20], citrate [21], a-amylase [22], alcohol fermentation [23], cheese fermentation [24], in food industry is well established. Entrapment of microorganisms in natural polymers such as alginate is favoured [25] for its simple, continuous operational ability and stability. Double layer immobilization in alginate prevents leaking of cells [26] thus prevents contamination, also it helps in enhancing survival [12] and thus prolonging the activity of microbial cells while permitting exchange of substrates, products, inhibitors, etc. Current study involved immobilizing six different bacterial species in double layer alginate by drop forming method and checking their efficacy in improving oil quality by reducing the rancidity level.

Rancidity is the characteristic, unpleasant odour and flavour of edible fats and oils following oxidative or hydrolytic degradation. In addition to this, rancidity may give rise to toxic levels of certain products e.g., aldehydes, hydroperoxides and epoxides [27]. The two well-known pathways that lead to rancidity are oxidation and hydrolysis. Oxidation leads to oxidative rancidity and involves oxygen attack of glycerides which can be initiated by heat, peroxidants, certain enzymes or light. During frying thermo-oxidative or lipid oxidation and hydrolytic reactions take place that result in deterioration in quality of the frying oil [28]. The primary oxidation products that develop in triacylglycerol are hydroperoxides, which may later break down to produce lower molecular

weight compounds, such as free fatty acids, alcohols, aldehydes, and ketones, eventually leading to a rancid product [1-2]. Peroxides are unstable under frying conditions. Peroxide values increase after sample is removed from the fryer, particularly during the cooling period, where the frying oil is exposed to air at high temperature [29]. The presence of air and water accelerates the deterioration of frying oil [30]. Lipid oxidation is a main deteriorative process which has an important implication in stipulations of the quality and value of fats and oils, particularly in relation to the off-flavours that develop as an outcome of autoxidation [31]. It also causes health hazard, like biological damage of living tissues and increase in the risk of cardiovascular diseases [32], diarrhoea and poor growth rate [33].

During deep-fat frying, FFA analyses are quality indicators that determine the amount of hydrolysis. Due to hydrolysis, hydrolytic rancidity occurs as a result of splitting of the triglyceride molecule at the ester linkage with the formation of free fatty acid (FFA) and other products. The rate of hydrolysis development is due to amount of moisture in the foods being friend and the frying temperature [34]. This decomposition results in poor performance of frying oil and off-flavour formation and may influence nutritional quality and food safety. Since repeated use may lead to health hazards, these cooking oils were considered for treatment with immobilized bacterial consortia.

The results revealed a positive outcome in reducing the rancidity with immobilized *S. aureus*, *P. aeruginosa*, *Bacillus spp II* and *Aeromonas sp*p during the current study. Similar results of lowering hydroperoxides [35] and peroxides using *S. aureus*, *Bacillus cereus*, *Micrococcus cryophilus* [36], removal of free fatty acids by *Pseudomonas* spp, reduction of peroxide value and FFA in animal related products like meat by *Clostridium* spp [37], removal of peroxides and carboxyl compounds from chicken tissues [38], removal of carboxyl compounds and hydroperoxides in rancid oil and fish waste [39] were observed where the activity of individual bacterial cultures had been considered.

However, current study focuses on reducing free fatty acid and Peroxide value of used cooking oils to improve their quality by reducing the rancidity. Yet complete mechanism for such effectiveness is yet to be established. One possible reason of reduction in FFA value can be that some microorganisms are able to utilize free fatty acids from the medium for their growth [40] or microorganisms producing specific lipases to esterify FFA with DAG [41-42]. Most likely reason for the decrease of the PV might be the release of antioxidative substances by the microorganisms or picking up active O_2 from the environment. Unsaturated fatty acids which are highly susceptible to peroxidation might be delayed due to treatment with microorganisms. This study makes clear that the use of microorganisms reduces peroxide value and FFA from the rancid vegetable oil thereby availing for reuse.

V. CONCLUSION

Quality of oil is reduced by oxidative and hydrolytic degradation accelerated due to frying, longer storage, heat exposure or moisture content. Improving PV and FFA value can be considered for improving the quality of used oil for reuse purposes. In this study we observed that the colour, viscosity and odour of the rancid oil were changed by the microorganisms. Although the decomposed products were not identified, it was evident that the immobilized microorganisms were able to bring down the peroxide and free fatty acid values of used cooking oil (Safflower oil, Groundnut oil) significantly. Immobilization of bacterial cells suggests better methodology as it prevents tedious processes of removal of cellular biomass from target sample and also helps prevent the contamination by leaking. This method can prove to be effective at commercial eateries where buying fresh oil is economically not convenient. Isolation and characterization of the ant oxidative substance along with the cytotoxicity and utility of this technique can be considered for further study.

REFERENCES

[1] Rani, A. K. S., Reddy, S. Y., & Chetana, R., "Quality changes in trans and trans free fats/oils and products during frying" *European Food Research and Technology*, vol *230*(6), pp 803-811, 2010.

[2] Singh, S., Gamlath, S., & Wakeling, L., "Nutritional aspects of food extrusion: a review", *International Journal of Food Science & Technology*, vol *42*(8), pp 916-929, 2007.

[3] Stevenson, S. G., Vaisey-Genser, M., & Eskin, N. A. M., "Quality control in the use of deep frying oils", *Journal of the American Oil Chemists Society*,vol *61*(6), pp 1102-1108, 1984.

[4] Perjési, P., Pintér, Z., Gyöngyi, Z., & Ember, I., "Effect of rancid corn oil on some onco/suppressor gene expressions in vivo. A short-term study" *Anticancer research*, vol *22*(1A), pp. 225-230, 2001.

[5] Halliwell, B., "Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis", *British journal of experimental pathology*, vol *70*(6), pp 737, 1989.

[6] Lazar, I., Petrisor, I. G., & Yen, T. E., "Microbial enhance recovery (MEOR)", *Petroleum Science and Technology*, vol 25, 11-12, 2007.

[7] McGenity, T. J., Folwell, B. D., McKew, B. A., & Sanni, G. O, "Marine crude-oil biodegradation: a central role for interspecies interactions" *Aquat. Biosyst*, vol *8*(10), pp 10-11, 2012.

[8] Antoniou, E., Fodelianakis, S., Korkakaki, E., & Kalogerakis, N, "Biosurfactant production from marine hydrocarbon-degrading consortia and pure bacterial strains using crude oil as carbon source", *Frontiers in microbiology*, vol *6*, 2015.

[9] *Dedhia C. and Jayaprada Rao Chunduri, "Isolation, Identification and characterization of oil degrading marine bacteria from sandy shore of shivaji park, Mumbai".* European journal of pharmaceutical and medical research ISSN 3294-3211; vol 2(5), pp 541-555, 2015.

[10] Bidoia, E. D., Montagnolli, R. N., & Lopes, P. R. M., "Microbial biodegradation potential of hydrocarbons

evaluated by colorimetric technique: a case study" *Current research, technology and education topics in applied microbiology and microbial biotechnology. Badajoz, Spain: Formatex*, pp. 1277-1288, 2010.

[11] Yamagiwa, K., Shimizu, Y., Kozawa, T., Onodera, M., & Ohkawa, A., "Formation of calcium-alginate gel coating on biocatalyst immobilization carrier" *Journal of chemical engineering of Japan*, vol *25*(6), pp. 723-728, 1992.

[12] Mokarram, R. R., Mortazavi, S. A., Najafi, M. H., & Shahidi, F. "The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice" *Food Research International*, vol *42*(8), pp. 1040-1045, 2009.

[13] Hoffpauir, C. L., Petty, D. H., & Guthrie, J. D.,"Germination and Free Fatty Acid in Individual Cotton Seeds" *Science (New York, NY)*, vol *106*(2754), pp. 344-345, 1947.

[14] Alimentarius, C. (1999). Codex standard for named vegetable oils. *Codex Stan*,*210*, 1-13.

[15] Das, N., & Chandran, P, "Microbial degradation of petroleum hydrocarbon contaminants: an overview" *Biotechnology research international*,*2010*.

[16] Hanson, K. G., Desai, J. D., & Desai, A. J, "A rapid and simple screening technique for potential crude oil degrading microorganisms" *Biotechnology techniques*, vol *7*(10), pp. 745-748, 1993.

[17] Montagnolli, R. N., Lopes, P. R. M., Cruz, J. M., Tamada, I. S., & Bidoia, E. D, "Biodiesel and vegetable oils in environment: biodegradation and toxicity through colorimetric analysis" In *Industrial, Medical and Environmental Applications of Microorganisms: Current Status and Trends: Proceedings of the V International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld2013) Mad* (p. 46). Wageningen Academic Publishers, May 2014.

[18] Mariano, A. P., Bonotto, D. M., Angelis, D. D. F. D., Pirôllo, M. P. S., & Contiero, J, "Biodegradability of commercial and weathered diesel oil".*Brazilian Journal of Microbiology*, vol *39*(1), pp. 133-142, 2008.

[19] Jobanputra, A. H., Karode, B. A., & Chincholkar, S. B, "Calcium alginate as supporting material for the immobilization of rifamycin oxidase from Chryseobacterium species" *Biotechnology, Bioinformatics and Bioengineering,vol 1*(4), pp 529-535,2011.

[20] Stenroos, S. L., Linko, Y. Y., & Linko, P., "Production of L-lactic acid with immobilized Lactobacillus delbrueckii. *Biotechnology Letters*, vol *4*(3), 159-164, 1982.

[21] Kautola, H., Rymowicz, W., Linko, Y. Y., & Linko, P. (1991). "Production of citric acid with immobilized Yarrowia lipolytica. *Applied microbiology and biotechnology*, *35*(4), 447-449.

[22] Ramakrishna, S. V., R. Jamuna, and A. N. Emery. "Continuous production of thermostable α-amylase by immobilized bacillus cells in a fluidized-bed reactor." *Applied biochemistry and biotechnology*, vol 37(3), pp. 275-282, 1992.

[23] Nagashima, M., Azuma, M., Noguchi, S., Inuzuka, K., & Samejima, H.,"Continuous ethanol fermentation using immobilized yeast cells", *Biotechnology and bioengineering*, vol *26*(8), pp 992-997, 1984.

[24] Prevost, H., & Divies, C. "Fresh fermented cheese production with continuous pre-fermented milk by a mixed culture of mesophilic lactic streptococci entrapped in Ca-Al ginate", *Biotechnology Letters*, vol *9*(11), 789-794, 1987.

[25] Klein, J., Stock, J., & Vorlop, K. D., "Pore size and properties of spherical Ca-alginate biocatalysts", *European journal of applied microbiology and biotechnology*, vol *18*(2), pp 86-91, 1983.

[26] Milagre, H. M., Milagre, C. D., Moran, P. J., Santana, M. H. A., & Rodrigues, J. A. R., "Asymmetric Bioreduction of Ethyl 3-Halo-2-oxo-4-phenylbutanoate by Saccharomyces c erevisiae Immobilized in Ca-Alginate Beads with Double Gel Layer", *Organic process research & development*, vol *10*(3), 611-617, 2006.

[27] Robards, K., Kerr, A. F., & Patsalides, E., "Rancidity and its measurement in edible oils and snack foods. A review" *Analyst*, vol *113*(2), pp 213-224, 1988.

[28] Perkins, E. G., "Effect of lipid oxidation on oil and food quality in deep frying", In *ACS Symposium series-American Chemical Society (USA)*, 1992.

[29] Gray, J. I., "Measurement of lipid oxidation: a review", *Journal of the American Oil Chemists' Society*, vol *55*(6), 539-546,1978.

[30] Clark, W. L., & Serbia, G. W., "Safety aspects of frying fats and oils", *Food Technology*, vol *45*(2), 84-89, 1991.

[31] Yu, X., Van De Voort, F. R., & Sedman, J., "Determination of peroxide value of edible oils by FTIR spectroscopy with the use of the spectral reconstitution technique", *Talanta*, vol *74*(2), 241-246, 2007.

[32] Addis, P. B., & Park, S. W. (1989). Role of lipid oxidation products in atherosclerosis.

[33] Sanders, T. A. B. (1983). Nutritional significance of rancidity. *Rancidity in foods/edited by JC Allen and RJ Hamilton*.

[34] O'brien, R. D. (2008). Fats and oils: formulating and processing for applications. CRC press, pp *219 – 221*.

[35] Updegraff, D. M., & Bovey, F. A. "Reduction of organic hydroperoxides by micro-organisms and animal tissues", *Nature*, vol *181*, pp 890-893, 1958.

[36] Lilly, H. D., Smith, J. L., & Alford, J. A., "Reduction in peroxide values and monocarbonyls of oxidized methyl oleate by several microbial cultures", *Canadian journal of microbiology*, vol *16*(9), 855-85, 1970.

[37] Yetim, H., Kayacier, A., Kesmen, Z., & Sagdic, O. (2006). The effects of nitrite on the survival of Clostridium sporogenes and the autoxidation properties of the Kavurma" *Meat science*, vol *72*(2), pp. 206-210, 2011.

[38] Moerck, K. E., & Ball Jr, H. R, "Influence of microorganisms on the carbonyl compounds of chicken tissue" *Journal of agricultural and food chemistry*, vol *27*(4), pp. 854-859, 1979.

[39] Harun-Or-Rashid, M., Kato, F., & Murata, A, "Effects of microorganisms on the peroxidation of lipid and fatty acid composition of fermented fish meal" *Bioscience, Biotechnology, and Biochemistry*, vol *56*(7), pp. 1058-1061, 1992.

[40] Cho, S. Y., Kwon, T. W., & Yoon, S. H, "Selective removal of free fatty acids in oils using a microorganism" *Journal of the American Oil Chemists' Society*, vol *67*(9), pp. 558-560, 1990.

[41] Bhosle, B. M., & Subramanian, R. "New approaches in deacidification of edible oils—a review" *Journal of Food Engineering*, vol *69*(4), pp. 481-494, 2005.

[42] Bhattacharyya, A. C., & Bhattacharyya, D. K., "Deacidification of high FFA rice bran oil by reesterification and alkali neutralization" *Journal of the American Oil Chemists' Society*, vol *64*(1), pp. 128-131, 1987.