Chemical and Enzymatic Valourisation of Confectionery Waste into Biofuel: An Application of Circular Economy

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INTRODUCTION

Confectionery industries contribute immensely towards global food supply (Henricus, 1980). These industries are major enterprises across the world and still expanding rapidly. Liquid and solid wastes generated from confectionery industries contain substantial amount of lipids and carbohydrates. These are non-edible in nature and needs to be managed from the circular economy perspective (Karmee, 2016a, 2016b, 2017, 2018a, 2018b; Karmee et al., 2015, 2018). Thus, these wastes can be valorised for biofuel production. In this context, majority of the world’s energy needs are met by burning natural gas, petroleum based fuels and coal. This is leading to depletion of fossil fuels and simultaneously causing environmental degradation. Therefore, there is a need for development of alternative energy resources. In addition, energy starved nations are also exploring renewable energy sources as an option to become energy independent. Along this line, biofuels are extensively researched and recognized as a potential source of energy.

In the above context, biodiesel is a commercially available fuel primarily synthesized from fats, oils and greases. Thus far, industrial biodiesel production is largely focussed on use of many edible feedstocks. However, use of edible feedstocks is creating several challenges including food vs fuel debate. In addition, feedstocks are also increasing the biodiesel cost. To circumvent these limitations, non-edible waste feedstocks viz. food waste, sewage sludge and organic waste are currently employed as low-cost or no-value resources for biofuel production (Karmee and Chadha, 2005; Karmee and Lin, 2014). In line with this, confectionery products and their wastes contain oils, syrups, nuts, candied fruits and colour powders. Confectionery industrial effluent streams containing oils and carbohydrates can be directly valorised for bioenergy production (Figure 1).

In this paper, biodiesel was prepared from a lollipop effluent stream. In the initial stage, oil from lollipop effluent stream was extracted using n-hexane and ethyl acetate. Approximately, 10-18 wt.% oil was recovered from lollipop effluent streams. Moisture from the obtained oil was removed using sodium sulphate (Na2SO4). Subsequently, for biodiesel production chemical (KOH, CaO and Ca(OH)2) and biocatalysts (lipases) were used under solvent free condition. Biodiesel preparation via lipase catalysis is advantageous since it is substrate specific, moisture tolerant, recyclable and operates under mild experimental conditions (Karmee 2015, 2016a, 2016b, 2017). Initially, a series of commercially available lipases were screened for biodiesel production from confectionery waste oil. Immobilized lipase from Candida antarctica lipase-B (CAL-B) was found to be suitable. Further optimization of reaction parameters were carried out using CAL-B. Key parameters including feedstock to alcohol molar ratio, temperature, and time course of reaction were optimised. Finally, reusability experiments for the Novozyme-435 was performed.
Figure 1. Plausible pathways for biofuel production from confectionery wastes

### METHODOLOGY

**Enzymes, Chemicals and Instruments**

Feedstock used in this experiment was procured from a local lollipop unit located in North-West province, South Africa. KOH, CaO, Ca(OH)$_2$, methanol, diethyl ether and deuterated chloroform (CDCl$_3$) of analytical grades were purchased from Sigma-Aldrich and Associated Chemical Enterprises (ACE), Johannesburg, South Africa. *Muco miehei*, *Pseudomonas cepacia*, *Rhizopus delemar*, *Geotrichum candidum*, *Candida rugosa*, *Caprina papaya*, *Porcine pancreas-II*, *Pseudomonas fluorescence* and *Candida antarctica* lipase-B (Novozyme-435) lipases were obtained from Sigma-Aldrich, Amano Enzyme Inc., Nagoya, Japan and from local enzyme suppliers of South Africa. Waste oil to biodiesel conversion (%) was calculated from $^1$H NMR (Nuclear Magnetic Resonance) using a Bruker instrument (600 MHz) located at the Faculty of Natural Sciences, Laboratory for Analytical Services, North West University, Potchefstroom, South Africa.

**Preparation of Biodiesel from Lollipop Effluent Using Base Catalysts (KOH, CaO and Ca(OH)$_2$)**

A reaction mixture containing waste oil (1 g) and methanol (480 μl) was prepared in a round bottom flask. To this mixture base catalyst (1 wt.%), was added at 60°C. The reaction was performed at 1:10 molar ratio of waste oil to methanol for 90 min. The reaction was monitored using $^1$H NMR.

**Screening of Different Lipases for Biodiesel Production from Lollipop Effluent**

Nine lipases as stated in the earlier section were used during the screening experiments. For the screening experiments a molar ratio 1:4 (oil to methanol) was used. The reaction mixture comprising methanol (189 μl), oil (1 g) and lipase (0.1 g, 10 wt.%) was stirred at 40°C for 6 h. After that, the mixture was diluted using diethyl ether (2 ml x 2). Subsequently, lipase was filtered and air dried. The biodiesel was placed in a 50 ml round bottomed flask and dried at 70°C for 30 min under vacuum. The product was analyzed using $^1$H NMR.

**Optimization of Feedstock to Alcohol Molar Ratio**

All experiments were started by addition of oil (1 g) to Novozyme-435 (0.1 g, 10 wt.%). Then methanol of required molar ratios were added (1:1 (47 μl), 1:3 (146 μl), 1:4 (189 μl), 1:5 (240 μl), 1:6 (285 μl), 1:8 (379 μl), 1:10 (480 μl) for optimization purpose at 40°C. Each reactions were carried out for 6 h. Downstream processing and $^1$H NMR analysis were carried out as mentioned in the earlier section.

**Optimization of Reaction Temperature**

A moral ratio of 1:4 (oil to methanol) was used for the experiments, which consists of a mixture of methanol (1:4,189 μl), feedstock (1g) and biocatalyst (Novozyme-435) (0.1 g, 10 wt.%). Reactions were performed at 30°C, 40°C, 50°C and 60°C to determine the maximum biodiesel yield. Each of these experiments was carried out for 6 h.

**Two-step Methanol Addition Process for Biodiesel Production**

A two-step methanol addition process was followed to maximize the yield of biodiesel. The mixture was comprised of methanol (1:4, 189 μl), oil (1 g) and biocatalyst (0.1 g, 10 wt. %). The initial time duration was 6 h at 40°C. Subsequently, after 6 h of duration an additional amount of methanol (47 μl) was added to the reaction flask through septum. Further, the reaction was allowed to continue until 36 h. All the obtained samples were analyzed using $^1$H NMR to quantify the biodiesel.

**RESULTS AND DISCUSSION**

**Confectionary Waste Utilization**

Different solid and liquid effluents are generated in confectionery industries, which are known to contain organic substances (Beal and Raj, 2000; Das et al., 2013; El-Kassas et al., 2015; Lafitte-Trouque and Forster, 2000). These wastes are generally composed of biodegradable materials such as sugar, sweetener, casein, oil, milk, food colouring and flavouring agents. These are considered no-value resources as these are discarded without any further applications. Confectionery wastes can be converted into aquatic feeds, carbon rich-sources, bioenergy and value added products (Beal and Raj...
### Table 1. Value added products from confectionery waste as compiled and reported by Johnstone-Robertson (2017)

| Solid waste                                                                 | Treatment technologies                                      | Product(s)                                                                 |
|------------------------------------------------------------------------------|------------------------------------------------------------|---------------------------------------------------------------------------|
| Confectionery waste                                                         | Biogas integrated gasification fuel cell                   | Electricity                                                               |
| Confectionery waste                                                         | Microbial cultivation                                      | Single cell protein (SCP)                                                 |
| Confectionery waste products                                                | Two-stage anaerobic digestion (AD)                         | Acetic acid, lactic acid, Ethanol and CO₂                                  |
| Flour waste streams                                                         | Batch fermentation                                          | Bacterial cellulose                                                       |
| Flour rich waste stream                                                      | Batch fermentation                                          | Poly hydroxyl butyrate (PHB)                                              |
| Flour rich waste                                                           | Fed-Batch fermentation                                     | Microbial lipid                                                           |
| Sweets                                                                       | Fuel cell                                                  | Electricity                                                               |
| Waste wafer material                                                        | AD                                                        | Biogas and digestate                                                      |

| Liquid waste                                                                | Treatment technologies                                      | Product(s)                                                                 |
|------------------------------------------------------------------------------|------------------------------------------------------------|---------------------------------------------------------------------------|
| Confectionery factory effluent                                              | Shake flask                                               | Algal biomass                                                             |
| Confectionery waste water                                                  | Sludge waste treatment plant                              | Water for irrigation                                                      |
| Confectionery waste water                                                  | Aerobic conditions                                        | Xanthan gum                                                               |
| Confectionery waste water                                                  | Anaerobic digestion (AD) with enrich H₂ producing bacteria | (Methanogens inactive)                                                   |
| Chocolate waste water                                                       | Microbial fuel cells                                       | Electricity                                                               |
| Chocolate waste water (Containing sugar syrups)                             | Dual anaerobic co-digestion                               | C₂H₄                                                                      |
| Chocolate waste water                                                       | AD                                                        | Biogas and COD reduction                                                  |
| Candyed jujube waste                                                        | Batch fermentation                                         | Bacterial cellulose                                                       |
| Confectionery waste water                                                  | Fermentation                                              | Bioflocculant                                                             |
| Confectionery waste water                                                  | Sloping pilot plant                                       | Microalgae biomass, enzymatic and non-enzymatic antioxidant               |
| Chocolate waste water                                                       | AD                                                        | Electricity                                                               |
| Confectionery waste water                                                  | Anaerobic pretreatment and aerobic treatment             | CH₄ and reduced COD levels and water for irrigation                       |
| Confectionery waste water                                                  | Sequential two-stage anaerobic treatment                  | Decrease in COD levels                                                    |
| Confectionery waste water                                                  | Fermentation                                              | Clean water with reduced COD, BOD, FOG, TSS, and odors.                   |
| Confectionery waste water                                                  | Aerobic treatment using trickling filter                  | Decrease in COD levels                                                    |

Figure 2. Distribution of different types of wastes generated in South Africa (Ekelund and Nyström, 2007; Environment outlook, 2013; NOWCS South Africa, 2015)

2000; Das et al., 2013; El-Kassas et al., 2015; Lafitte-Trouque and Forster, 2000). In this context, South Africa, has many confectionery industries such as lollipop factories that produce sizeable quantities of waste, which can be valorised into biofuels, chemicals and biopolymer (García et al., 2011; Genc and Ozbay, 2015; Gough et al., 2013; Johnstone-Robertson, 2017; Lunghi and Burzacca, 2004; Pilarska et al., 2019; Ranade et al., 1989; Ruggeri et al., 2013; Tsakona et al., 2016) (Table 1). Similarly, using advanced valorisation technologies the ~ 15% organic waste generated in South Africa can be converted into biofuels, chemicals, materials and other high value products (Ekelund and Nyström 2007; NOWCS South Africa 2015; Environment outlook 2013). (Figure 2). Environmental friendly management of confectionery wastes is significant from waste management and circular economy context (Miah et al., 2018) (Figure 3).

**Biodiesel Production from Lollipop Effluent via Base Catalysis**

Food industries generate significant amounts of waste during processing, production, packaging, and storage (Sohair et al., 2008). South Africa generates large quantities of organic food wastes which needs technical valorisation (Greben and Oelofse, 2009; Oelofse and Nahman, 2015). During the course
of this work, lollipop industry waste stream was valorised for biofuel production. Initially oil from the lollipop effluent was extracted using n-hexane and ethyl acetate (95v: 5v) as a solvent. Up to 10-18 wt.% of oil was recovered from confectionery waste samples. After extraction, the recovered oily portions were combined and concentrated via a rotary evaporator. To remove trace amount of moisture in the oily fraction it was dried over sodium sulphate and stored for further use.

The waste oil isolated from the lollipop effluent was subjected to base catalysed biodiesel preparation. The KOH, CaO and Ca(OH)_2 catalysed (1 wt.%) reactions were performed at 1:10 molar ratio of waste oil to alcohol at 60 °C in a two necked round bottom flask equipped with heating bath, condenser and magnetic stirrer (Figure 4). Among all base catalysts, high conversion (99%) of biodiesel was observed in 20 min during KOH catalysed reaction (Figure 5).

**Biodiesel Production from Lollipop Effluent Using Lipases**

Lipases are widely used enzymes for applications in energy, food, beverage, detergent, leather, pharmaceutical and paper industries. Under physiological conditions, lipases catalyse hydrolysis of triglycerides to glycerol and free fatty acids. However, in non-conventional media lipase catalyses esterification and transesterification reaction. As mentioned in Figure 6, nine different lipases were screened for biodiesel production from waste oil derived from lollipop effluent stream. The biodiesel yield was in the range of 4-34% (Figure 6). During screening it was observed that *Candida antarctica* lipase-B (Novozyme-435) gave 34% biodiesel yield (Figure 6).

Novozyme-435 was applied for further optimization of reaction conditions. The time course study of biocatalytic biodiesel production was carried out for 36 h. However, at 18 h, a maximum 94% biodiesel yield was achieved (Figure 7). Therefore, 18 h is considered to be the optimum reaction time. The reusability of the biocatalyst (Novozyme-435) was studied at 1:5 molar ratio and 40 °C. It was found that, after 14th reaction cycle, 72 % biodiesel yield was obtained. Around 24% decrease in biodiesel yield was observed after the 14th reaction cycle (Figure 8).

Base catalysed reaction is faster as compared to the enzyme catalysed reactions. During base catalysis methoxide is formed; whereas, during lipase catalysed reaction enzyme-
substrate complex is formed. For the base catalysed reaction optimum conditions are: 1:10 molar ratio (substrate to alcohol), 60°C and 20 min (reaction time). In contrast, for the lipase catalysed reaction, optimum conditions are: 1:5 molar ratio (substrate to alcohol), 40°C and 18 h.

CONCLUSIONS

Liquid effluents from confectionery industries are potential feedstocks for biofuel production. In this work, both chemical (base) and biocatalysts (lipases) were used for biodiesel preparation from confectionery waste oil. KOH gave 99% biodiesel yield in 20 min; whereas, Novozyme-435 catalyzed reaction produced 94% biodiesel in 18 h.

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REFERENCES

Beal, L. J. and Raj, R. D. (2000). Sequential two-stage anaerobic treatment of confectionery wastewater. *Journal of Agricultural Engineering Research, 76*, 211-217. https://doi.org/10.1006/jaer.2000.0555

Das, B. K., Gauri, S. S. and Bhattacharya, J. (2013). Sweetmeat waste fractions as suitable organic carbon source for biological sulphate reduction. *International Biodeterioration & Biodegradation, 82*, 215-223. https://doi.org/10.1016/j.ibiod.2013.05.027

Ekelund, L. and Nyström, K. (2007). Composting of municipal waste in South Africa - sustainability aspects. Uppsala University, Sweden. Retrieved from https://www.utn.uu.se/sts/student/wp-content/uploads/gamla%20exjobb/0602_kristinanystromlottenekelund.pdf (Accessed: 11 March 2020).

El-Kassas, H. Y., Heneash, A. M. M. and Hussein, N. R. (2015). Cultivation of Arthrospira (Spirulina) platensis using confectionery wastes for aquaculture feeding. *Journal of Genetic Engineering and Biotechnology, 15*(2), 145-155. https://doi.org/10.1016/j.jgeb.2015.08.003

Environment outlook: Chapter 13, *Waste management*, South Africa. (2015). Retrieved from https://www.environment.gov.za/sites/default/files/reports/environmentoutlook_chapter13.pdf (Accessed: 11 March 2020).

García, I. L., Dorado Pérez, M. P., López, J. A., Villar, M. A., Yanniotis, S. and Koutinas, A. (2011). Valorisation of confectionery industry wastes for the microbial production of polyhydroxalkanoates. *International Congress on Engineering and Food, Food process engineering in a changing world*, vol. II, 745-746, Atenas, Greece, 22-26.

Genc, N. and Ozbay, I. (2015). Fermentative hydrogen production in batch experiments using molasses, potato processing industry wastewater and chocolate waste: influence of acidic hydrolyzation. *Asian Journal of Chemistry, 27*(6), 2184-2188. https://doi.org/10.14233/ajchem.2015.18059

Gough, H. L., Nelsen, D., Muller, C. and Ferguson, J. (2013). Enhanced methane generation during thermophilic co-digestion of confectionery waste and grease-trap fats and oils with municipal wastewater sludge. *Water Environment Research, 85*(2), 175-183. https://doi.org/10.2175/106143012X15418552642128

Greben, H. A. and Oelofse, S. H. H. (2009). Unlocking the resource potential of organic waste: a South African perspective. *Waste Management & Research, 27*(7), 676-684. https://doi.org/10.1177/0734242X09103817

Henricus, A. J. M. D. (1980). Method for making confectionery lollipops. US 4208437, Application number, US 05/950,122.

Johnstone-Robertson, M. (2017). Value recovery from confectionery waste. Retrieved from https://www.wastero admap.co.za/download/ims2017_foodwaste_present15.pdf (Accessed: 11 March 2020).

Figure 7. Reaction kinetics of Novozyme-435 catalysed biodiesel production from confectionery waste

Figure 8. The reusability of Novozyme-435 during biodiesel preparation
Karmee, S. K. (2015). Lipase catalyzed synthesis of fatty acid methyl esters from crude Pongamia oil. *Energy Sources Part A, 37*, 536-542. https://doi.org/10.1080/15567036.2011.572131

Karmee, S. K. (2016a). Liquid biofuels from food waste: current trends, prospect and limitation. *Renew Sustain Energy Rev*, 53, 945-955. https://doi.org/10.1016/j.rser.2015.09.041

Karmee, S. K. (2016b). Preparation of biodiesel from nonedible plant oils using a mixture of used lipases. *Energy Sources Part A*, 38(18), 2727-2733. https://doi.org/10.1080/15567036.2015.1098748

Karmee, S. K. (2018a). A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. *Waste Management*, 72, 240-254. https://doi.org/10.1016/j.wasman.2017.10.042

Karmee, S. K. (2018b). Enzymatic biodiesel production from Manilkara Zapota (L.) seed oil. *Waste Biomass Valorization*, 9, 725-730. https://doi.org/10.1007/s12649-017-9854-8

Karmee, S. K. (2020). Technical valorisation of spent coffee grounds and food waste using sulfuric acid immobilized on silica. *Biofuels*, 11(2), 155-161. https://doi.org/10.1080/17597269.2017.1378989

Karmee, S. K. and Chadha, A. (2005). Preparation of biodiesel from crude oil of Pongamia pinnata. *Bioreour Technol*, 96, 1425-1429. https://doi.org/10.1016/j.biortech.2004.12.011

Karmee, S. K. and Lin, C. S. K. (2014). Lipids from food waste as feedstock for biodiesel production: Case Hong Kong. *Lipid Technol*, 26, 206-209. https://doi.org/10.1002/lite.201400044

Karmee, S. K., Linardi, D., Lee, J. and Lin, C. S. K. (2015). Conversion of lipid from food waste to biodiesel. *Waste Manage*, 41, 169-173. https://doi.org/10.1016/j.wasman.2015.05.025

Karmee, S. K., Wian, S. and Marx, S. (2018). Biofuel production from spent coffee grounds via lipase catalysis. *Energy Sources Part A*, 40(3), 294-300. https://doi.org/10.1080/15567036.2017.1415394

Lafitte-Trouque, S. and Forster, C. F. (2000). Dual anaerobic co-digestion of sewage sludge and confectionery waste. *Bioreour Technol.*, 71, 77-82. https://doi.org/10.1016/S0960-8524(99)00045-7

Lunghi, P. and Burzacca, R. (2004). Energy recovery from industrial waste of a confectionery plant by means of BIGFC plant. *Energy*, 29(12-15), 2601-2617. https://doi.org/10.1016/j.energy.2004.05.016

Miah, J. H., Griffiths, A., McNeill, R., Halvorson, S., Schenker, U., Espinoza-Orias, N. D., Morse, S., Yang, A. and Sadhukhan, J. (2018). Environmental management of confectionery products: Life cycle impacts and improvement strategies. *J. Clean. Prod.*, 177(10), 752-751. https://doi.org/10.1016/j.jclepro.2017.12.073

NOWCS, The national organic waste composting strategy, South Africa. (2013). http://sawic.environment.gov.za/documents/1825.pdf (Accessed: 10 March 2020).

Oelofse, S. H. H. and Nahman, A. (2013). Estimating the magnitude of food waste generated in South Africa. *Waste Manag Res.*, 31(1), 80-86. https://doi.org/10.1177/0748414712472765

Oias, R., Billard, D., Dukuck, F., Kookos, I., Wian, S., Ibarra, P. and Zaborowicz, M. (2019). Use of confectionery waste in biogas production by the anaerobic digestion process. *Molecules*, 24(1), 37. https://doi.org/10.3390/molecules24010037

Pilarska, A. A., Pilarski, K., Wolna-Maruwka, A., Boniecki, P. and Zaborowicz, M. (2019). Use of confectionery waste in biogas production by the anaerobic digestion process. *Molecules*, 24(1), 37. https://doi.org/10.3390/molecules24010037

Ranade, D. R., Yeole, T. V., Meher, K. K., Gadre, R. V. and Godbole, S. H. (1989). Biogas from solid waste originated during biscuit and chocolate production: a preliminary study. *Bioreour Technol.*, 28(2), 157-161. https://doi.org/10.1016/0269-7488(89)90079-7

Ruggeri, B., Luongo Malave, A. C., Bernardi, M. and Fino, D. (2013). Energy efficacy used to score organic refuse pretreatment processes for hydrogen anaerobic production. *Waste Manage, 33*(11), 2225-2233. https://doi.org/10.1016/j.wasman.2013.06.024

Sohair, I. A., Fayza, A. N. and Saber, A. E. (2008) Wastewater management in small- and medium-size enterprises: case studies. *Environmentalist*, 28, 289-296. https://doi.org/10.1007/s10701-007-9142-4

Tsakona, S., Skiadariess, A. G., Kopsahelis, N., Chatzifragkou, A., Papanikolaou, S., Kookos, I. K. and Koutrina, A. A. (2016). Valorisation of side streams from wheat milling and confectionery industries for consolidated production and extraction of microbial lipids. *Food Chem, 198*, 85-92. https://doi.org/10.1016/j.foodchem.2015.11.031