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EFFECT OF PROCESS PARAMETERS ON BIODIESEL PRODUCTION FROM EDIBLE AND WASTE OILS BY *BURKHOLDERIA CEPACIA* LIPASE

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INTRODUCTION

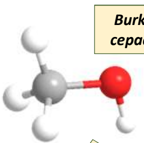
Although biodiesel has been on the market for many years as an alternative to fossil fuels and its production is carried out through the already known routes of chemical transesterification, the need for development of more environmentally friendly production using lipase is still in the focus. To gain insight into the effects of several process parameters on the biocatalytic biodiesel production, we have characterised *Burkolderia cepacia* lipase and used it in the production of biodiesel from edible and waste cooking oils and fats at previously optimised pH and *T*. In addition, characterization of the feedstock including fatty acid profile, percentage of free fatty acids, peroxide and iodine value was performed.

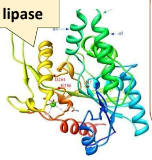
MATERIALS & METHODS

Edible and waste oils

Fatty acid profile (GC-FID)
Free fatty acids [%] (ISO 660)
Peroxide value [mmol O_2/kg] (ISO 3690)
Iodine value [g I_2/100 g] (ISO 3961)

Burkholderia cepacia lipase





Methanol

pH and temperature optimum (spectrophotometric assay with *p*-nitrophenyl palmitate as substrate) and substrate specificity (titrimetric assay)

BIODIESEL SYNTHESIS
V=250 mL
Lipase activity load 250 U/g
850 rpm
50°C/24 h

Fatty Acid Methyl Esters (FAME) analysis after 1, 3, 6 and 24 h of reaction (GC-FID)

Table 1 Free fatty acid, peroxide and iodine value of selected waste cooking oils (WCO), waste cooking fat (WCF) and sunflower oil (SFO)

Samples	WCO 1	WCO 2	WCO 3	WCF	SFO
FFA [% of oleic acid]	0.37 ± 0.02	2.92 ± 0.04	0.23 ± 0.02	0.51 ± 0.01	0.17 ± 0.01
PV [mmol O_2/kg]	3.98 ± 0.33	1.29 ± 0.26	1.40 ± 0.42	1.60 ± 0.42	0.39 ± 0.02
IV [g I_2/100 g]	123.88 ± 2.41	116.68 ± 2.11	120.34 ± 3.23	74.72 ± 2.89	119.43 ± 1.04

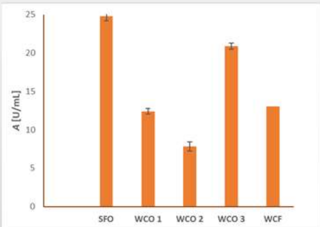


Figure 2 Substrate specificity of *Burkholderia cepacia* lipase toward selected waste cooking oils (WCO), waste cooking fat (WCF) and sunflower oil (SFO).

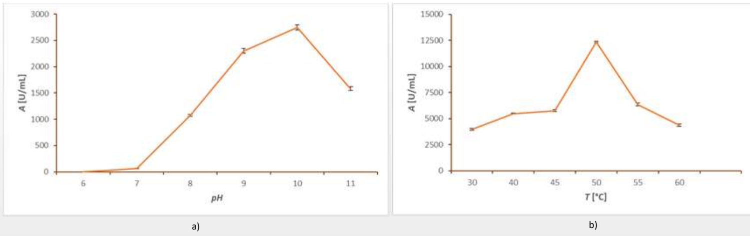


Figure 1 a) pH optimum of *Burkholderia cepacia* lipase; b) temperature optimum of *Burkholderia cepacia* lipase

Table 2 Fatty acid profile of selected waste cooking oils (WCO 1-3) and waste cooking fat (WCF).

Samples	C14:0	C16:0	C16:1	C18:0	C18:1n9c+t	C18:2n6*c*	C18:2n6t	C18:3n3	C20:0	C20:1	C22:0	C24:0	Total fatty acids [%]
WCO 1	-	5.87±0.01	0.14±0.01	2.94±0.01	30.70±0.08	58.6±0.00	-	0.10±0.01	0.21±0.01	0.12±0.00	0.61±0.01	0.17±0.05	99.43±0.03
WCO 2	-	6.87±0.07	0.14±0.01	1.90±0.04	43.77±0.06	41.43±0.19	-	3.28±0.02	0.29±0.00	0.50±0.00	0.30±0.00	-	98.47±0.01
WCO 3	-	6.27±0.04	0.14±0.01	3.00±0.02	32.90±0.00	55.86±0.01	0.12±0.01	0.10±0.00	0.21±0.01	0.14±0.01	0.60±0.00	0.15±0.08	99.47±0.01
WCF	1.73±0.31	23.07±0.56	3.57±0.23	7.48±0.01	46.18±1.25	12.77±0.28	-	0.66±0.01	-	1.23±0.05	-	-	96.67±0.08

Table 3 Time-dependent FAME profile and total yield during biodiesel synthesis at 50 °C by *B. cepacia* lipase from waste cooking oils (WCO), waste cooking fat (WCF) and sunflower oil (SFO)

Samples	WCO 1				WCO 2				WCO 3			
Time [hr]	1	3	6	24	1	3	6	24	1	3	6	24
C14:0	0.09±0.01	0.08±0	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0	0.09±0.01	0.08±0.01	-	-	-	-
C16:0	7.19±0.49	8.22±2.16	7.97±2.14	7.38±1.30	7.36±0.04	8.39±0.69	8.72±1.24	8.12±0.83	7.06±0.20	7.11±0.26	7.04±0.16	6.90±0.04
C16:1	0.13±0.05	0.16±0.01	0.17±0.04	0.16±0.02	0.13±0	0.14±0.01	0.14±0.01	0.13±0.01	0.13±0	0.13±0	0.13±0	0.13±0.01
C18:0	2.90±0.05	3.07±0.12	3.18±0.31	3.14±0.24	1.88±0.02	2.13±0.15	2.12±0.09	2.04±0.12	3.09±0.04	3.21±0.12	3.17±0.09	3.10±0.06
C18:1n9c+t	28.98±1.08	28.51±1.35	29.09±1.67	29.36±1.26	42.29±0.26	40.60±0.58	40.39±0.83	41.54±1.14	31.09±0.77	30.94±0.91	31.37±0.61	32.10±0.14
C18:2n6c	59.18±0.66	57.34±2.70	56.76±2.74	57.90±0.67	41.84±0.23	40.93±1.29	40.20±2.42	40.70±1.21	55.90±0.31	56.01±0.23	55.86±0.18	55.44±0.11
C18:2n6t	-	-	-	-	-	-	-	-	0.10±0	0.10±0.01	0.10±0	0.11±0.01
C18:3n3	0.11±0	0.11±0.01	0.11±0.01	0.12±0.03	0.11±0	0.11±0.01	0.11±0.01	0.12±0.03	0.11±0.01	0.11±0.01	0.11±0.01	0.11±0.01
C20:0	0.15±0.02	0.15±0.04	0.14±0.06	0.18±0.02	0.15±0.02	0.15±0.04	0.14±0.06	0.18±0.02	0.19±0.01	0.20±0.01	0.20±0.01	0.20±0
C20:1	0.11±0.01	0.12±0.01	0.13±0.02	0.12±0	0.11±0.01	0.12±0.01	0.13±0.02	0.12±0	0.15±0	0.15±0.01	0.14±0.01	0.15±0.01
C22:0	0.41±0.06	0.42±0.06	0.45±0.11	0.51±0.10	0.41±0.06	0.42±0.06	0.45±0.11	0.51±0.10	0.57±0.02	0.60±0.02	0.59±0.01	0.61±0.11
C24:0	0.08±0	0.09±0	0.13±0	0.14±0	0.08±0	0.09±0	0.13±0	0.14±0	0.10±0.08	0.15±0.01	0.14±0.01	0.16±0.11
TOTAL FAME [%]	99.17±0.15	98.09±1.98	98.00±2.11	98.89±0.60	99.17±0.15	98.09±1.98	98.00±2.11	98.89±0.60	98.49±0.46	98.70±0.35	98.85±0.24	99.01±0.10

Samples	WCF				SFO			
Time [hr]	1	3	6	24	1	3	6	24
C14:0	0.72±0.04	0.55±0.03	0.45±0.02	-	-	-	-	-
C16:0	8.33±0.43	5.75±0.19	4.93±0.34	9.23±1.81	8.57±.01	8.66±0.41	8.04±0	6.50±0.19
C16:1	3.26±0.09	3.49±0.13	2.91±0.09	5.12±0.27	0.11±0.03	0.11±0.04	0.10±0.01	0.09±0.01
C18:0	5.68±0.06	3.93±0.08	3.41±0.53	3.47±0.69	3.80±0.04	4.13±0.06	3.97±0.31	3.12±0.12
C18:1n9c+t	54.41±1.27	56.66±1.10	51.12±2.16	44.82±1.32	31.00±0.08	30.78±0.77	30.95±0.11	31.26±0.16
C18:2n6c	15.25±0.68	16.06±0.23	18.14±1.26	32.44±1.96	54.44±0.29	54.26±0.24	55.19±0.24	57.24±0.05
C18:2n6t	-	-	-	-	-	-	-	-
C18:3n3	0.69±0.03	0.75±0.01	0.81±0.05	-	-	-	-	-
C20:0	0.22±0.01	0.20±0.01	0.27±0.01	-	0.15±0	0.16±0.01	0.15±0.01	0.13±0.01
C20:1	1.59±0.06	1.67±0.01	1.65±0.23	-	-	-	-	-
C22:0	-	-	-	-	0.56±0.01	0.65±0.11	0.68±0.02	0.71±0
C24:0	-	-	-	-	-	-	-	-
TOTAL FAME [%]	90.15±1.06	89.06±1.15	83.69±2.03	92.63±2.26	99.30±0.47	99.3±0.15	99.5±0.02	99.5±0.01

RESULTS AND DISCUSSION

The characterization of *B. cepacia* lipase was performed in order to select the optimal pH (10) and temperature (50 °C) conditions for the biodiesel synthesis (Figure 1). After biodiesel synthesis, independently on the oil used SFO/WCOs, it could be noticed that more than 98% of FAME was produced after the first hour of transesterification reaction which fully meets European standard (FAME ≥ 96.5%) (Table 3). Considering WCF, it can be noted that after the first hour there is a conversion of about 90%, which after 24 hr increases to approximately 92%. Therefore, it can be concluded that when using WCF, the minimum for FAME yield required by the Standard cannot be met. According to the determined fatty acid profile (Table 2) WCOs and WCF, and known fatty acid profile of SFO, it is not surprising that the largest share of FAME was formed from unsaturated long chain fatty acids C18:2 and C18:1 and saturated C16:0 since these fatty acids were the most abundant in examined oils and fat. Greater than 99% of FAME is produced after one hour when using SFO and WCO 1. These results were expected since *B. cepacia* lipase showed highest substrate specificity toward these two oils (Figure 2). Also, according to determined characteristic (Table 1) WCO 3 actually corresponded to fresh sunflower refined oil.

CONCLUSION

The results showed that at optimized process conditions after one hour of reaction time, 250 U of the lipase per 1 g of reaction 99% when fresh edible oil was used as feedstock, while 97% of FAME was reached when waste cooking oil/fat was used as feedstock. Iodine value, as one of the indicators of oil/fat quality, showed statistically significant correlation with fatty acid methyl esters content, what indicates that it can be used as one of the indicators for selecting feedstock for enzymatic production of biodiesel.n mixture was sufficient to achieve fatty acid methyl ester content

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