Production of Medium Chain Fatty Acids From Ensiled Potato Peels; Effect of Inoculum Type and Kinetic Study

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ABSTRACT: Medium chain fatty acids (MCFAs) are fatty acids containing 6 to 12 carbon atoms with a wide range of industrial application. They can be produced by the fermentation of waste biomass through a process called chain elongation (CE). During CE, the type of inoculum used plays a key role in determining the optimal yield of MCFAs. In this study, we showed, for the first time, the use of three different inocula including leachate, rumen fluid and digestate from a biogas reactor for the batch fermentation of ensiled potato peels for MCFAs production. Results showed that the highest chain elongation was obtained when leachate was used as inoculum with a maximum yield of 57, 4 and 26 g/kgVS for caproic acid, heptanoic acid and caprylic acid respectively. A kinetic study shows that the production of MCFAs from ensiled potato peels was better described by the first-order model than by the modified Gompertz model.

KEYWORDS: Batch fermentation; Chain elongation; Electron donors; Lactic acid; Waste biomass.

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I. INTRODUCTION

In order to reduce the impact of fossil fuel combustion on man and the environment, the production of alternative fuels and fuel-based products from renewable sources has continued to receive global attention from researchers. Some of such fuel-based products are medium chain fatty acids (MCFAs), which are saturated fatty acids with carbon chain lengths from 6 to 12, and can be used as additives in animal feed, antimicrobial agents and lubricants, with a projected market price of about 8 billion USD by 2023 (Leeuw *et al*., 2019). At the moment, the majority of MCFAs are produced from non-sustainable sources; fossil fuels and food crops (Ren *et al*., 2022). However, MCFAs can be produced by the fermentation of biomass through a process called chain elongation (CE). During CE, electron donors (ED) such as lactic acid and/or ethanol are used to elongate short chain fatty acids, SCFAs (electron acceptors, EA) through reverse β-oxidation in the presence of dedicated microorganisms (Wu *et al*., 2021).

MCFAs have been produced by the fermentation of synthetic EDs and EAs with yields that are 10 times higher than yields from organic sources (Groof *et al*., 2019). However, a life-cycle assesment shows that such processes have serious environmental consequences (Chen *et al*., 2017). Environmental safety and economics of CE can be enhanced if the substrates to be used are non-toxic to the environment, readily available, and contain the required EDs and EAs such that supplementation is not required. Waste streams from alcohol (Ma *et al*., 2022) and milk (Coelho *et al*., 2020) production are some of such substrates. Another example of substrates that contain inherent EDs and EAs is an ensiled material. During ensiling, the water soluble carbohydrates in a substrate are converted to fermentation products like lactic acids, ethanol and acetic acids (Undiandeye *et al*., 2022a) which are necessary for CE. In order to implement a circular economy, wastes that are common to a particular locality can be ensiled and used for the production of MCFAs. In Nigeria, potato peels (PP) are some of the wastes that are readily available and are usually a source of environmental pollution. Available studies on the fermentation of PP has produced mainly short chain fatty acids, SCFAs (Iglesias-Iglesias *et al*., 2021; Lu *et al*., 2020), probably because the substrate has limited EDs and EAs required for CE. The concentration of these EDs and EAs can be improved by ensiling.

The type of inoculum used for CE and their physical structure also plays a major role in determining the yield of MCFAs (Atasoy *et al*., 2019). For instance, Atasoy *et al*. (2020) reported a significant difference in the yield of fatty acids from the fermentation of cheese whey in the presence of three different inocula. Inocula from reactors producing biogas (Sarkar *et al*., 2021), treating wastewater (Wu *et al*., 2020) and from rumen samples (Villegas-Rodríguez and Buitrón, 2021) have been widely used, with the type of substrate also influencing the yield of MCFAs.

In a previous study (Kefas and Undiandeye, 2022), PP were ensiled for biogas production. The ensiled PP contained a significant amount of lactic acid, acetic acid and ethanol. In the present study, the aim is to investigate the potential of the ensiled PP as a substrate for MCFAs production using three different inocula. In order to understand the dynamics of the

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overall performance of the process, a kinetic evaluation was also carried out to determine process parameters that could be used for the control and optimization of the fermentation of PP for MCFAs production.

II. MATERIALS AND METHODS

A. Substrate and Inocula

The substrate used was PP that has been previously ensiled for 100 days. The properties of the silage are outlined in Table 1 (Kefas and Undiandeye, 2022). Three different inocula were used for the present study including leachate from a waste collection point (A), cattle rumen fluid collected from an abattoir (B) in Port Harcourt, and digestate from the study of Kefas and Undiandeye (2022) (C). The properties of the inocula are also listed in Table 1. Upon collection, all inocula were stored at 4 °C until required.

Table 1. Physicochemical properties of substrate and inocula.

			Inocula		
Property	Unit	PP	A	B	C
TS	%	12.71 ± 0.18	-	-	-
VS	%TS	94.37 ± 2.37	-	-	-
BOD	g/L	-	8250 ± 11	58 ± 2	2168 ± 8
COD	g/L	-	12307 ± 23	97 ± 5	6217 ± 13
pH	-	3.79 ± 0.01	4.37 ± 0.21	6.94 ± 0.47	7.97 ± 0.83
AA	g/kgFM	4.51 ± 0.01	-	-	-
LA	g/kgFM	6.83 ± 0.17	-	-	-
Eth	g/kgFM	2.73 ± 0.08	-	-	-

TS, total solids; VS, volatile solids; BOD, biochemical oxygen demand; COD, chemical oxygen demand; AA, acetic acid; LA, lactic acid; Eth, ethanol; A, leachate; B, rumen fluid; C, digestate.

B. Batch Fermentation

Fermentation was carried out in 200 mL serum bottles (Figure 1), each with a working volume of 125 mL. The initial pH in each reactor was kept at 5.5 ± 0.2 based on the study of Lu *et al*. (2020). Feeding of reactors with substrate and inoculum was done inside an anaerobic chamber in order to make the systems anaerobic. Each reactor contained a substrate to inoculum ratio (S/R) of 3:1 (gVS basis). A S/R of 3:1 allows for a fast acclimatization of acidogenic bacteria to the CE process as well as inhibits methanogens (Perimenis *et al*., 2018). Anoxic distilled water was used to make up the stated working volume. The desired pH was attained by the addition of 10 M HCl or 10 M NaOH solution. After feeding and pH adjustment, the bottles were sealed with butyl rubber stoppers and aluminium caps, and incubated for 28 days at a mesophilic temperature of 32 ± 2 °C based on the study of Xu *et al*. (2021).

C. Analytical Methods

Total solids (TS), volatile solids (VS), pH, organic acids and ethanol concentration were measured using standard methods as previously described (Kefas and Undiandeye, 2022). Total solids and volatile solids were corrected using appropriate equations in order to avoid overestimation of product yields. The head space gas composition in each bottle was measured by withdrawing 1 mL of gas sample using a syringe and then transferred into 20 mL glass vials that had been flushed with argon. Measurement was then carried out using gas chromatography equipped with an autosampler in a Perkin Elmer GC. The yield (Y), productivity (P) and selectivity (S) of each MCC was calculated using Eqns. 1, 2 and 3 respectively.

$$Y = \frac{cv}{m_s} \quad (1)$$

$$P = \frac{c}{t} \quad (2)$$

$$S = \frac{c}{c_T} \quad (3)$$

where c = maximum concentration of a given MCC (g/L), v = working volume of reactor (L), m_s = mass of added substrate (kg VS), t = time at which maximum c was achieved (d), c_T = total concentration of carboxylates in the fermentation broth (g/L).

D. Kinetic Modelling of Medium Chain Carboxylates Production

Two kinetic models were used to fit the production of medium chain carboxylates in order to improve the understanding of the process. These models were the first-order model and the modified Gompertz model given in Eqns. 4 and 5 respectively.

$$c_t = c_{max}[1 - \exp(-k \times t)] \quad (4)$$

$$c_t = c_{max} exp\left\{-exp\left[\frac{R_{max}e}{c_{max}}(\lambda - t) + 1\right]\right\} \quad (5)$$

where c_t = concentration of a carboxylate at any time (g/L), t; cmax = maximum concentration of carboxylic acid (g/L); k = kinetic constant (/d); R_{max} = maximum rate of carboxylic acid production (g/L/d), λ = lag phase (d).

The Solver add-in tool in Microsoft Excel was used to estimate the parameters of each model.

E. Statistical Analysis

All data were analysed using analysis of variance (ANOVA) in OriginPro software (OriginLab corporation, Northampton, USA). Tukey's test was used to compare significant difference at 95% confidence level. Two statistical parameters including the coefficient of determination (R^2) and the Akaike Information Criterion (AIC) Eqn. 6 were used to determine the model that gave a better description of a given MCFA production.

$$AIC = N \times LN\left(\frac{SS}{N}\right) + 2k \quad (6)$$

where N = number of experimental data, SS = sum square of residuals, k = number of parameters in a model.

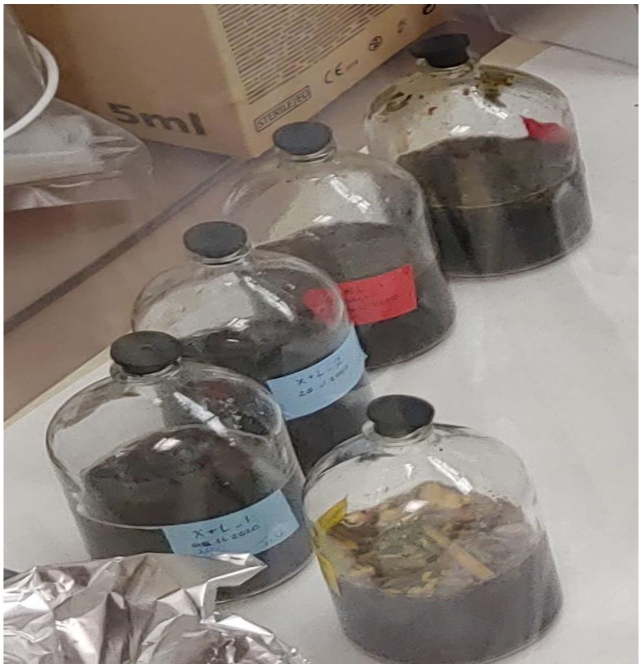

Figure 1. Reactors used for batch fermentation.

III. RESULTS AND DISCUSSION

A. *Effect of Inoculum Type on MCFAs Production*

The fermentation profile of fatty acids production in the presence of the three inocula is shown in Figure 2. Acetic acid was the dominant fatty acid in the systems as has also been reported by other authors (Saadoun *et al.*, 2022; Zhang *et al*., 2022), irrespective of the type of inoculum used. The high concentration of acetic acid during anaerobic fermentation has been attributed to the excessive oxidation of ethanol (Wu *et al*., 2021). There was a gradual increase in MCFAs production until day 21. Beyond that day, the concentration of MCFA reduced, probably due to depletion in nutrients and the experiment was therefore terminated. There was a general decrease in the pH of the systems after 7 days of fermentation due to the formation of SCFAs (Bolaji and Dionisi, 2017). Beyond day7, the pH stabilized until the end of the experiment, as also reported by Saadoun *et al*. (2022).

The concentration of butyric acid increased until day 3 and then decreased thereafter. This can be explained by the fact that CE is a stepwise process that begins with the elongation of acetic acid to butyric acid, butyric acid to caproic acid, and so on. During the first few days when caproic acid and other MCFAs were not formed, butyric acid accumulated in the systems. The concentration of butyric acid dropped from day7 when it was elongated to caproic acid and caprylic acid. The MCFAs produced were caproic acid, heptanoic acid and caprylic acid, with the highest concentration of these acids produced in the systems containing leachate as inoculum with a selectivity of 28%, 2% and 13% respectively after 21 days of fermentation. Unlike the report of Yebouet *et al*. (2021) in the fermentation of ethanol using leachate as inoculum, MCFAs with chain lengths greater than 8 were not detected. However, like in the present study, only MCFAs with chain lengths $\leq$ 8 were reported by Saadoun *et al*. (2022) in the fermentation of municipal solid waste with leachate as inoculum.

In the systems containing rumen fluid as inoculum, the concentration of caproic acid, heptanoic acid and caprylic acid was also significantly different ($p<0.05$) with a selectivity of 20%, 1% and 9% respectively. Villegas-Rodríguez and Buitrón (2021) also reported a significant difference in the concentration of these acids in the fermentation of ethanol and acetic acid using rumen fluid as inoculum. CE in the systems containing biogas digestate as inoculum was the least as seen from the low selectivity (caproic acid, 4%; heptanoic acid, 0.2%; caprylic acid, 1%) of MCFAs, which is in agreement with the report of Bolaji and Dionisi (2017) in the fermentation of vegetable and salad waste.

The yield and productivity of MCFAs in the presence of the three inocula are shown in Table 2. Clearly, digestate from a biogas reactor is not an appropriate inoculum for the fermentation of ensiled PP at the stated conditions. The fermentation of other mass flows like cheese whey (Jankowska *et al*., 2017) and diary wastewater (Silva *et al*., 2021) with digestate from biogas reactor as inoculum have also produced negligible yields of MCFAs.

B. *Biogas Composition*

In addition to the MCFAs, biogas was also produced in the reactors. Although the inocula were not heat-treated to deactivate methanogens, methane was not detected throughout the experiment. The absence of methane could be due to two factors; the pH at which the experiment was conducted and the S/R. The levels of these parameters (pH and S/R) used in the present study have been reported to inhibit methanogenes during CE (Perimenis *et al*., 2018). If methanogenes are not inhibited during CE, available EDs and EAs will be converted to methane and the yield of MCFAs will be significantly reduced. The gas produced was mainly composed of carbon (iv) oxide and hydrogen, with the highest composition of these gases obtained in the systems containing leachate (73.58% CO_2 and 26.42% H_2) on day 21. The high composition of H_2 is an indication that ensiled PP could also be a potential substrate for biohydrogen production.

C. *Kinetic Study*

The kinetic parameters of the models used to predict the production of MCFAs are shown in Table 3. Inoculum type had a significant effect ($p<0.05$) on the first-order kinetic constants, k, of each MCFAs. Although temperature is the most important factor that affects first-order constants (Fogler, 2016), other process parameters like pH and S/R can also influence it during anaerobic fermentation (Allaart *et al*., 2021; Fang *et al*., 2019). However, since all the systems were operated at the same temperature, S/R and pH, it is possible that the inoculum type may have been responsible for the difference in k. Kinetic constants of 0.057 /d and 0.321 /d have been reported for caproic acid production in the fermentation of swine wastewater (Morais *et al*., 2020) and diary wastewater (Coelho *et al*., 2020) respectively using the same inoculum, indicating that kinetic constants may also be specific to substrate type. Table 3 also shows that for all inocula, the first-order constants were highest for caproic acid production and

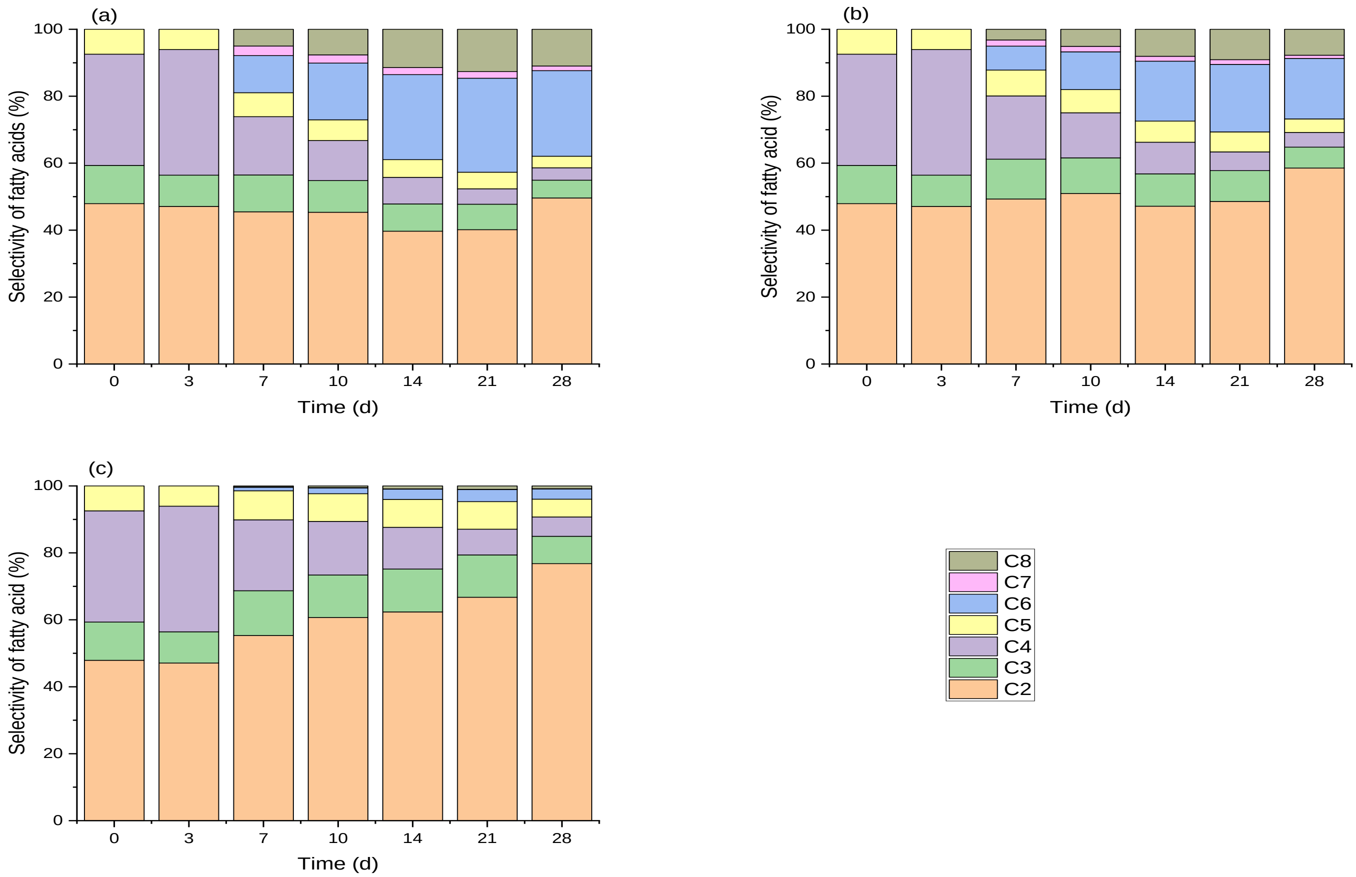

Figure 2. Fermentation profile of volatile fatty acids using (a) Leachate (b) Rumen fluid (c) biogas digestate, as inoculum (C2, acetic acid; C3, propionic acid; C4, butyric acid; C5, valeric acid; C6, caproic acid, C7, heptanoic acid; C8, caprylic acid).

Table 2. Mean values of Yield, productivity and selectivity (± standard deviation) of MCFAs

Inoculum	Parameter	Unit	C6	C7	C8
A	Yield	g/kgVS	57.32 ± 1.79	4.04 ± 0.13	25.79 ± 2.37
	Productivity	g/L/d	0.06 ± 0.00	0.004 ± 0.00	0.03 ± 0.00
B	Yield	g/kgVS	38.4 ± 0.92	2.70 ± 0.05	17.28 ± 0.14
	Productivity	g/L/d	0.04 ± 0.00	0.003 ± 0.00	0.02 ± 0.00
C	Yield	g/kgVS	4.59 ± 0.26	0.12 ± 0.00	1.29 ± 0.01
	Productivity	g/L/d	0.01 ± 0.00	0.0001	0.001

A, leachate; B, rumen fluid; C, digestate; C6, caproic acid; C7, heptanoic acid; C8, caprylic acid.

Table 3. Mean values of estimated parameters for the production of MCFAs.

				Inoculum		
Model	MCFA	Parameter	Unit	A	B	C
FOM	C6	k	/d	0.207	0.147	0.019
		c_{max}	g/L	1.351	0.978	0.107
	C7	k	/d	0.018	0.013	0.001
		c_{max}	g/L	0.088	0.063	0.005
	C8	k	/d	0.114	0.103	0.003
		c_{max}	g/L	0.561	0.496	0.030
MGM	C6	λ	d	6.1	5.8	6.4
		c_{max}	g/L	1.874	1.037	0.116
		R_{max}	g/L/d	0.089	0.049	0,006
	C7	λ	d	6.9	6.4	7.2
		c_{max}	g/L	0.103	0.082	0.007
		R_{max}	g/L/d	0.005	0,004	0.000
	C8	λ	d	7.3	7.1	7.4
		c_{max}	g/L	0.927	0.563	0.047
		R_{max}	g/L/d	0.044	0.027	0.002

lowest for heptanoic acid production, an indication of the ease of production of the respective acids. From the modified Gompertz model, the lag phase for the production of MCFAs was seen to be independent of inoculum type, probably because the same S/R was used, and were in agreement with the experimental data. Although an S/R of 3:1 allows for a fast acclimatization of acidogenic bacteria to CE process (Perimenis *et al*., 2018), the high lag phase shows that other factors like nature of substrate could also delay CE. For instance, using the same inoculum (brewery wastewater), lag phases of 6.9 d and 0.1 d were reported for caproic formation in the fermentation of swine wastewater and diary wastewater respectively (Coelho *et al*., 2020; Morais *et al*., 2020).

The maximum rate, R_{max}, of MCFAs was also obtained from the systems with leachate as inoculum and were in agreement with the experimental data. All kinetic models gave a reasonably good fit to the experimental data as seen from the high values of coefficient of determination ($R^2 \geq 0.907$).

However, the better model is the one with not just the higher R^2 value, but also with the smaller AIC value (Undiandeye *et al*., 2022b). Therefore, from Table 4, the first-oder model gave a better prediction of the experimental data than the modified Gompertz model.

Table 4. Statistical parameters describing the fittness of the models.

		First-order		Modified Gompert	
Inoculum	MCFA	R^2	AIC	R^2	AIC
	C6	0.991	-34.62	0.962	-27.81
Leachate	C7	0.987	-17.49	0.983	-12.73
	C8	0.942	-26.41	0.917	-18.83
	C6	0.998	-49.36	0.952	-35.69
Rumen Fluid	C7	0.995	-39.87	0.981	-31.87
	C8	0.934	-28.33	0.927	-26.59
	C6	0.993	-25.21	0.974	-23.47
Digestate	C7	0.961	-32.11	0.948	-25.46
	C8	0.996	-26.72	0.907	-17.89

The fitness of the better model (first-order) to the experimental data are shown in Figure 3. The shape of the curves gives an insight on the performance of the microbial community and biodegradability of the substrate. The lag phase (day 0-7) of the microbial community for the formation of MCFAs is clearly seen to be the same as was determined from the modified Gompertz model.

A good engineering practice is to make the lag phase as short as possible. One way of doing this is by using an inoculum from a reactor producing MCFAs. Such an inoculum can easily acclimatize to the formation of the acids. Another phase that can be observed from the curves is the exponential phase. This phase is associated with higher substrate consumption, MCFAs production and growth of microbial community. The decline in MCFAs production is the last phase, which has been attributed to products toxicity, and nutrients depletion (Fogler, 2016).

IV. CONCLUSION

In this study, the effect of inoculum type on medium chain fatty acids (MCFA) production as well as the kinetics of their formation from ensiled potato peels was investigated. The inocula used were leachate, rumen fluid and digestate from a biogas reactor. We observed that ensiled potato peels could serve as a potential substrate for MCFA production. The highest yield of MCFA was obtained when leachate was used as inoculum while the lowest yield was obtained when digestate from a biogas reactor served as inoculum. In the anaerobic fermentation of ensiled potato peels with leachate as inoculum for MCFA production, two objectives can be simultaneously achieved; limiting the impact of the waste

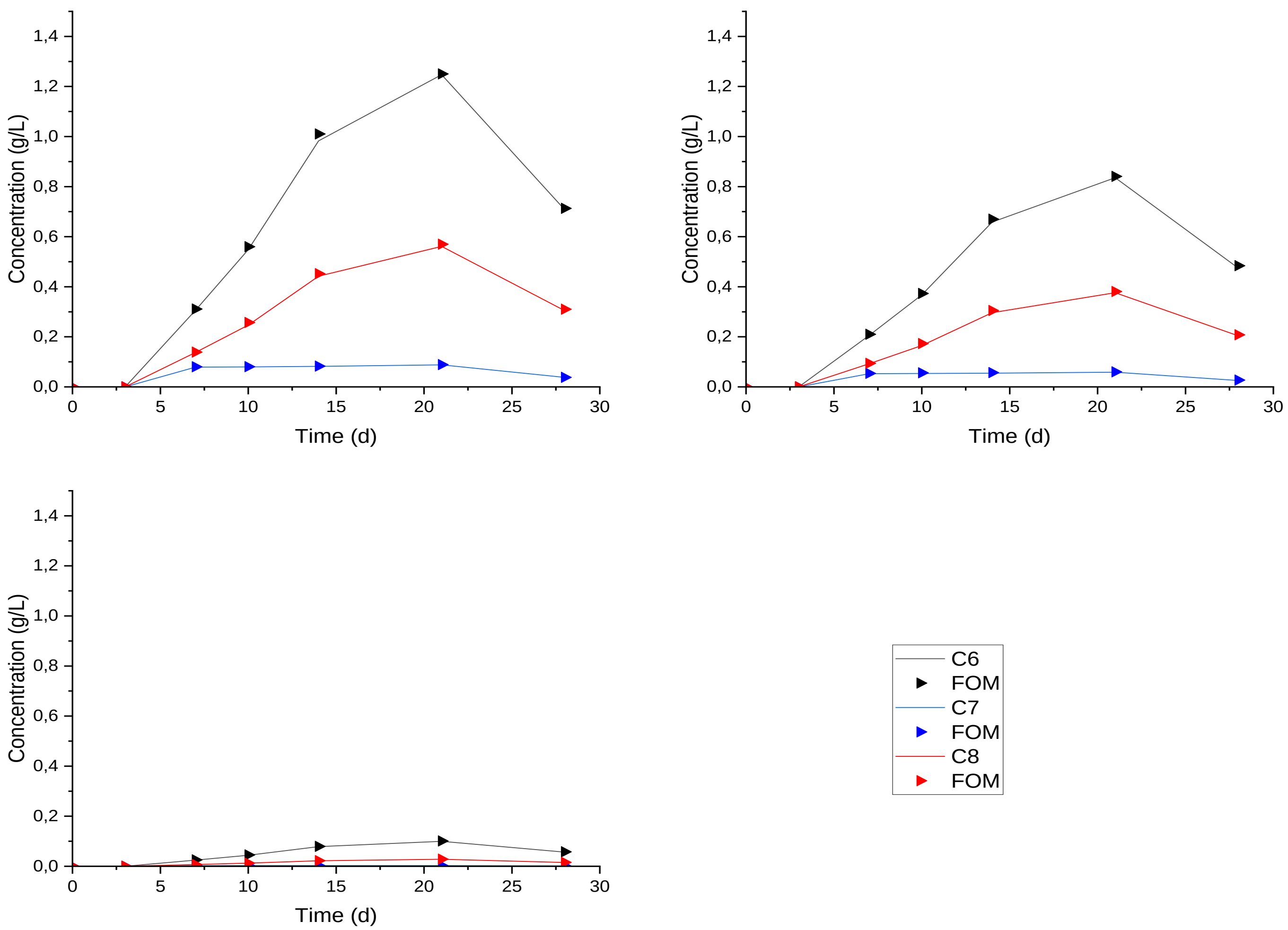

Figure 3. Kinetic fittings of the experimental data.

biomass on the environment and turning waste to wealth, thereby enhancing circular economy. However, the formation of MCFA was only observed after 7 days of fermentation irrespective of the inoculum used. This period could be shortened if an enriched inoculum like a fermentation broth from a reactor producing MCFA is used as inoculum. In designing a system for the continuos production of MCFAs from PP using leachate as inoculum, parameters from the first-order model can be applied.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the preparation of the manuscript.

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