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Biomethanation of white rotted and brown rotted rice straw

A. Ghosh, B.C. Bhattacharyya

Abstract Biomethanation of white rotted and brown rotted rice straw was taken for the present investigation and their efficiency on biomethanation has been tested. Rice straw was treated with white rot fungus *Phanerochaete chrysosporium* (P_C) and brown rot fungus *Polyporus ostreiformis* (P_O). Biogas and methane production was increased by about 34.73% and 46.19% in P_C-treated straw and 21.12% and 31.94% in P_O-treated straw respectively. VFA production has also been increased in P_C and P_O treated straw compared to control straw which were 76.73% and 30.69% respectively. Reduction of COD has also been found during biomethanation. The rate of reduction of COD during the initial period of digestion was 59.01%, 55.55% and 26.00% in P_C-treated, P_O-treated and control straw respectively after 21 days of digestion.

1
Introduction

The potential energy recovery from agricultural residues has been receiving great attention of energy planners and scientists. Biomethanation of agroresidues has several advantages over the other gasification process.

Lignocellulosic biomass are composed of lignin, cellulose and pentosan. Lignin in plant cell wall combines with carbohydrate to form lignin–carbohydrate complexes (LCC), Lignin–carbohydrate complexes show amphophatic or surface active properties due to the presence of hydrophilic carbohydrate and hydrophobic lignin in the same molecule. This lignin–carbohydrate complexes resist the plant cell wall to microbial attack. Therefore lignin degradation is the primary step for bioconversion of lignocellulose. There are various methods for pretreatment such as physical, chemical and biological methods for delignification. Lignins are degraded by aerobic microbes such as fungi, bacteria, actinomycetes, white rot basidiomycetes etc.

Biomethanation refers to a broad spectrum of biochemical process by which organic materials are upgraded and transformed into higher grade fuels and other products. Methane production from animal wastes mainly cattle dung and manure was studied first but field crop residues are the major potential energy sources and can be converted to methane under anaerobic digestion has been investigated [1, 2, 3]. Lignin is resistant to microbial decomposition but it can be metabolised aerobically [4].

Earlier workers also reported biogas production from plant materials pretreated with chemicals. Pretreatment of wheat straw by 1% NaOH for 7 days showed improved microbial digestibility and biodegradability during anaerobic fermentation at ambient temperature [5]. But biological methods for lignin degradation has seldom been attempted. Wheat straw was pretreated with Basidiomycetous fungus and observed biogas production from myco straw treated straw [6].

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2.1
Materials

2.1.1
The organisms

a) For delignification white rot fungus *Phanerochaete chrysosporium* (P_C) NCIM and brown rot fungus *Polyporus ostreiformis* (P_O) was used.
b) For methanation organisms were collected from cowdung which is enriched with *Methanococcus* sp., *Methanobacillus* sp. and *Clostridium* sp.

2.1.2
Growth media

For culture, fungus was maintained on 2% malt extract agar medium (Himedia, India). For pretreatment study locally collected rice straw (*Oryza sativa*) was used as the growing substrate which was soaked with Czapek Dox madium with primary C-source starch at 15% level.

2.2
Experimental methods

2.2.1
Inoculum preparation for pretreatment

For inoculum preparation the fungus was grown on 2% malt extract agar plates for 6 to 9 days at 38 ± 1 °C). Eight discs (dia. 1 cm each) containing 1.2 to 1.4 mg (dry wt) of mycelial mat were inoculated in 20 gm rice straw kept in polypropylene bags. This was done aseptically in a laminar air flow cabinet (Klenzaids, India).

2.2.2
Substrate preparation for delignification

Rice straw was chopped to a length of 0.7–1.0 cm (8–9% moisture content). Twenty grams of it was taken in poly-

propylene bag and soaked in sufficient tap water for overnight at room temperature (30 ± 1 °C). After draining away the excess water, moistened rice straw (having 65–70% water) was soaked in 45 ml Czapek Dox medium and sterilized in autoclave at 121 °C for 30 mins. The composition (in g/l) of the Czapek Dox medium was as follows – $NaNO_3 = 0.2$; $K_2HPO_4 = 1$; $MgSO_4 \cdot 7\ H_2O = 0.5$; $KCl = 0.5$; $FeSO_4 \cdot 7H_2O = 0.02$. Separately sterilized starch solution was added into it (15% w/v) as a primary growth substrate. Forty-five ml of this solution was added to 20 g rice straw which was inoculated with 8 mycelial discs (1 cm dia.) and incubated for 3 weeks under 88–90% relative humidity. Same procedure was followed for preparation of control untreated set except inoculation. After inoculation the contents of three replicated were dried at 105 °C, milled and analysed.

2.2.3
Experimental methods for biomethanation

Experimental set up for biomethanation is shown in Fig. 1. It consist of anaerobic bioreactor of capacity 5 liter (working volume 4 liter) as shown by item (A). The feed is introduced through a funnel (B) placed at the top of this digester. Leachate withdrawal port is provided at the bottom of the reactor as shown by item (C). Leachate recycling port (D) is connected with a peristaltic pump which is extended upto feeding funnel (B). One thermometer is inserted into digester as shown by item (E). Gas outlet pipe (F) is provided at the top of the digester and is connected with water reservoir (G and G′) for gas collection. The gas collector has an outlet pipe (H) at the top for gas collection and burning.

Anaerobic bioreactor (A) was filled with 8% total solid of 200 g delignified and control straw, 1.5 liter previously cultured inocular slurry and 1.0 liter water. The average working temperature was 30 °C and pH was maintained at 7.0–8.0. Digestion was carried out upto 63 days. The biogas produced was collected in a graduated water reservoir by displacement of saturated brine solution. The composition of biogas was analysed twice a week, pH of the digester slurry, volatile fatty acids (VFA) content in the digester and COD values of the digester leachate were recorded.

2.2.4
Analytical studies

Total carbon was estimated by Walkey and Black method [7] and total nitrogen by micro Kjeldahl method [8], cellulose was estimated by semi-micro method [9] and lignin by acetyl bromide method [10]. Small volume of digester liquid was taken for chemical analysis according to standard method. Pentosans were determined as "xylose equivalent" by hydrochloric acid distillation to furfural [11]. Total solid, volatile solid and ash content were measured by Standard methods of APHA [12]. Small volume of digester liquid was taken for chemical analysis according to Standard methods. Leachate was centrifuged at 5000 rpm for 5 mins and supernatant was used for analysis. Volatile fatty acids (VFA) as acetic acid was determined by distillation method of APHA [12]. COD were determined by Standard methods of APHA [12]. Gas composition was analysed by gas chromatography, equipped with a Porapak Q column, using thermal conductivity detector.

3
Results and discussion

The composition of rice straw is tabulated in Table 1. Effect of age of inoculum on lignin biodegradation is shown in Figs. 2 and 3 using P_C and P_O. It has been found from the Fig. 2 that 4 days old mycelia of P_C showed highest amount of lignin degradation compared to other age groups after 3 weeks of incubation. Similarly 8 days old mycelia of P_O showed a better result than others (Fig. 3). The loss of lignin is higher in P_C-treated straw which is 47.51% and in P_O-treated straw the respective value was 19.87% after 3 weeks of incubation as shown in Fig. 2. This delignified rice straw was used in anaerobic reactor for generation of biogas. During anaerobic fermentation gas was evolved. From Fig. 4, it has been found that gas pro-

Table 1. Composition analysis of rice straw

Components	wt%
Total solid[a]	91.03
Moisture[a]	8.97
Volatile solid[b]	81.09
Ash[b]	18.91
Total carbon	40.30
Total nitrogen	0.51
Cellulose	31.5
Pentosan	26.1
Lignin	14.9

[a] Total 100%
[b] Total 100%

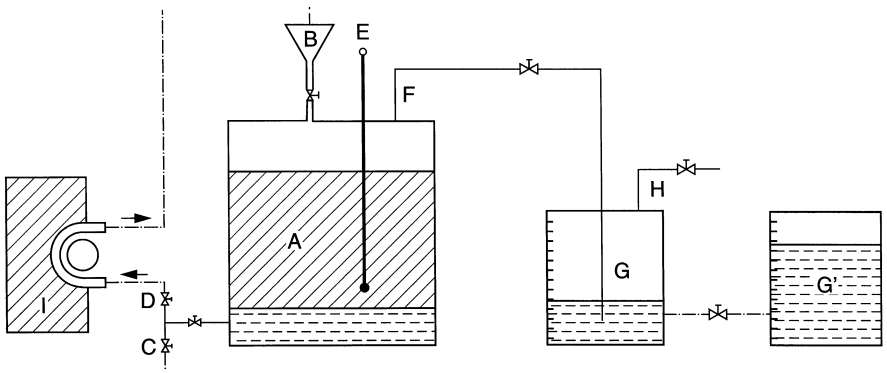

Fig. 1. Experimental set up for single stage biomethanation

duction was higher for pretreated straw than for control straw. In P_C and P_O-treated straw maximum amount of biogas and methane were 479.40 and 327.92 liter/kg dry biomass and 430.95 and 295.95 l/kg dry biomass and in control straw the respective values were 355.80 and 224.3 l/kg dry biomass. After removal of lignin the substrate cellulose and pentosans became more accessible to methanogens, therefore gas production was increased. The rate of gas production increased about 1.8–2.1 fold for P_C-treated and P_O-treated straw than for untreated control straw. The percentage of methane is also increased in delignified straw. The methane production is increased by 46.19% and 31.44% for P_C and P_O-treated straw compared to control straw.

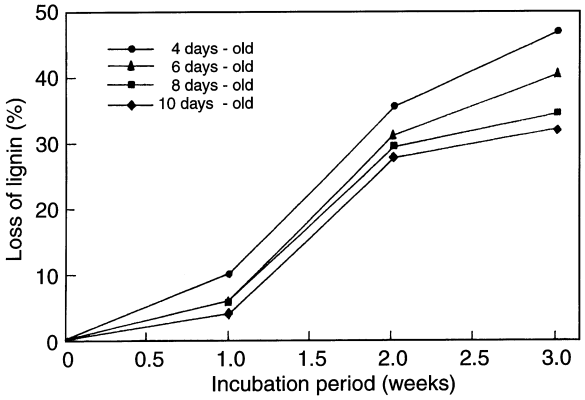

Fig. 2. Effect of age of inoculum on lignin biodegradation by P_C

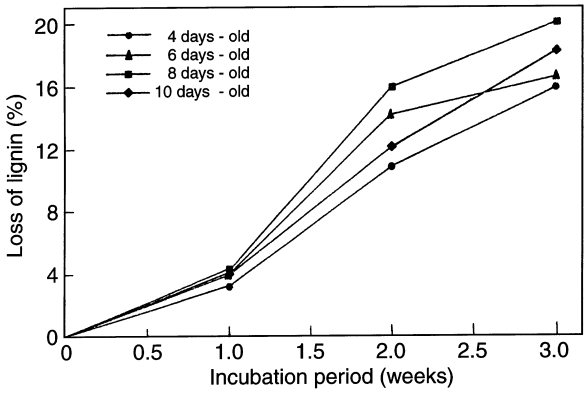

Fig. 3. Effect of age of inoculum on lignin biodegradation by P_O

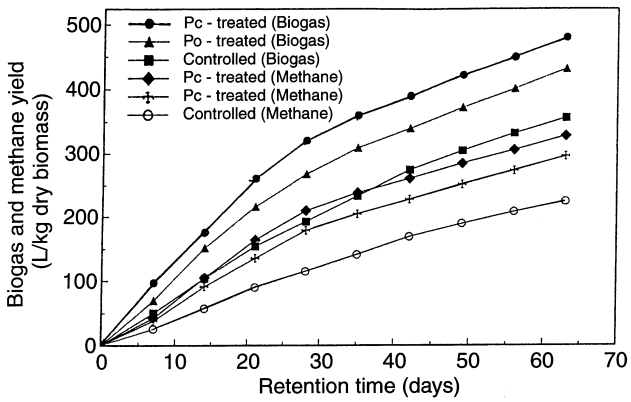

Fig. 4. Cumulative yield of biogas and methane

Samples of effluent from the digester were collected for the analysis of pH and volatile fatty acids (TVFA) as acetic acid concentration at various retention times. The results are shown in Fig. 5. From this figure it may be seen that volatile fatty acid concentration rises sharply within 7 days, falls slowly upto 21 days and thereafter reduction is rapid. The pH profile is also shown in Fig. 5. From the figure it is seen that the maximum of acid formation occurred for P_C-treated straw which is 3.57 g/liter within 7 days, but the respective values were 2.64 g/liter and 2.02 g/liter in case of P_O-treated and control straw respectively. A proper acid level gives rise to increase in specific growth rate of methanogens resulting in improved kinetics of methane formation [13]. Good biodegradability of millet bran is shown by the rapid VFA production [14]. It has been reported that plant materials with higher cellulose contents do not destroy the pH balance [1].

The leachate from the digester was collected at different retention times and analysed, the COD values are shown in Fig. 6. It is found from the figure that for P_C-treated and P_O-treated straw the COD was higher than that of control straw. The rate of decrease of COD is quite sharp during the initial period of 14 to 28 days for P_C and P_O-treated straw, whereas in control straw the decrease of COD is not very fast during the initial 21 days. The overall reduction of COD for P_C-treated, P_O-treated and control straw were 93.49%, 91.66% and 87.50% respectively, after 63-days of digestion. The initial rate of reduction of COD for

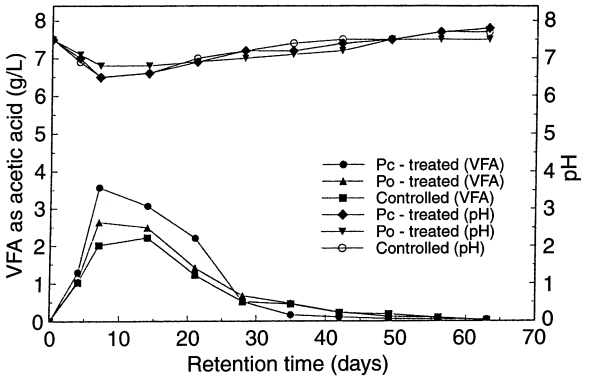

Fig. 5. VFA production and pH profile with retention time

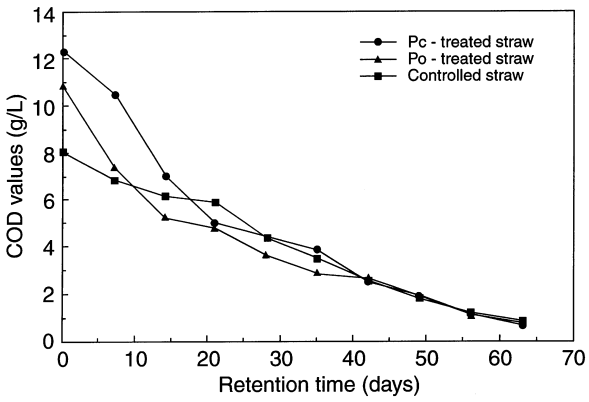

Fig. 6. Time courses of COD values with retention time

P_C-treated, P_O-treated and control straw were 59.01%, 55.55% and 26.00% respectively after 21 days of digestion (Fig. 7).

The leachate from the digester was collected at different time intervals and is shown in Fig. 8. It may be seen that the BOD_5 removal was 95.7%, 92.5% and 90.0% for P_C-treated, P_O-treated and control straw respectively after 63 days of digestion as shown in Fig. 9. From this it follows that the removal rate of BOD_5 (upto 35 days) was faster compared to that during the subsequent period.

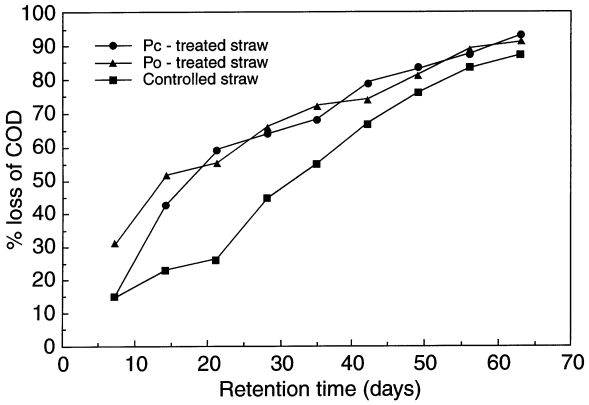

Fig. 7. Percent COD removed with retention time

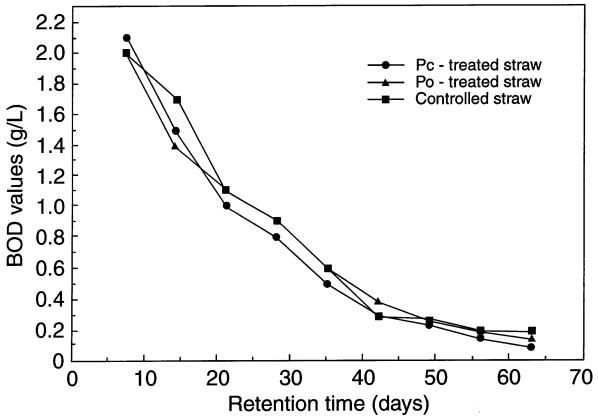

Fig. 8. BOD_5 values with retention time

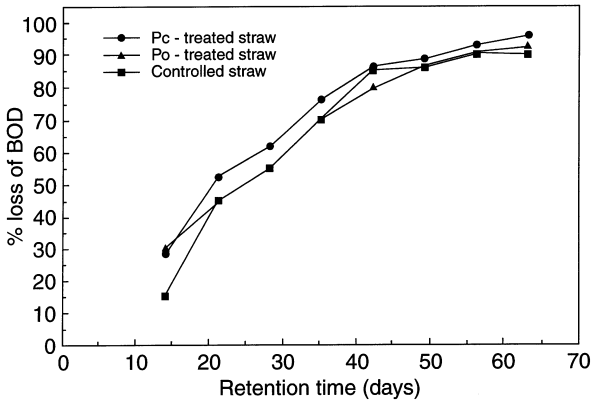

Fig. 9. Percent BOD_5 removed with retention time

It has been found from Fig. 10 that during the initial stage, biogas production is low due to the nonavailability of the substrates i.e. volatile fatty acids. Thereafter with the increase of VFA production, gas production also increases. For P_C-treated straw maximum amount of gas and acid production were 479.40 liter/kg dry biomass and 3.57 g/liter respectively. Similarly for P_O-treated straw and control straw these values were 430.95 liter/kg dry biomass and 2.64 g/liter and 355.80 liter/kg dry biomass and 2.02 g/liter respectively. The volatile fatty acid production was for longer duration for pretreated straw than for untreated straw. Therefore gas production is increased in pretreated straw.

Insoluble organic materials are degraded to form soluble organics which are utilized by methanogens. From Fig. 11 it is found that gas production increases with COD reduction. The only way by which COD reduction can be accounted for in an anaerobic digestion process, is through the removal of organic material from waste, such as by evolution of methane and carbon dioxide. Hence the total gas production is directly proportional to the COD reduction. It is also found from the figure that COD reduction increases with retention time.

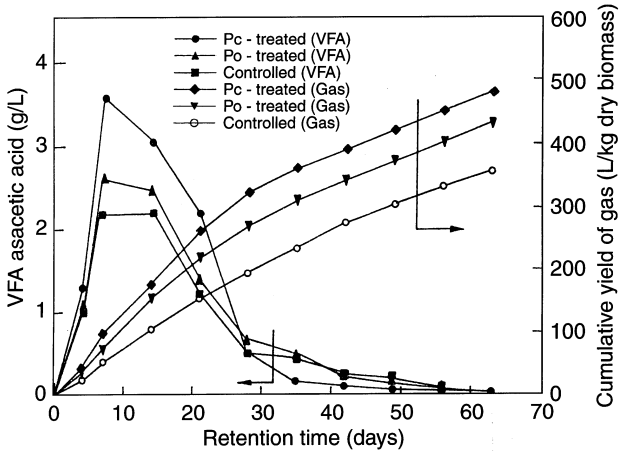

Fig. 10. Relation between gas evolution and volatile fatty acid degradation

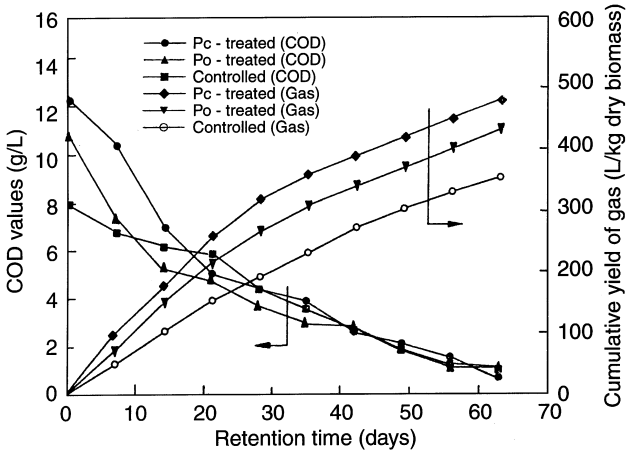

Fig. 11. Relation between COD utilization and biogas production

The above results have demonstrated the positive effect of hydrolysis on anaerobic biodegradability of rice straw. This is attributed to the fact that pretreatment of rice straw by white rot and brown rot fungi resulting in decreasing the association of lignin with carbohydrates and hence in increasing the substrate accessibility and anaerobic biodegradability. Metabolic activity of the organisms are also increased due to pretreatment.

This finding also supports Müller and Trösch's results [1986]. Their results showed that "myco-straw" can be better hydrolysed and converted to biogas in comparison to untreated wheat straw.

4
Kinetic model for substrates degradation

In the anaerobic digestion solid forms of biomass are converted into liquid forms by using hydrolyzing microbes. As a result liquid is enriched with carbon compounds which is designated as COD. These carbons are then converted into gaseous form such as methane and carbon dioxide by using microbes. Some amount of it is utilized for cell growth and maintenance. So, solid carbon particles are converted into gaseous carbon via liquid carbon which is the main substrates for the methanogenesis. The following major reaction steps are involved in the anaerobic digestion of solid biomass to methane:

Solid substrates utilization

$K \downarrow$ Hydrolyzing microbial action

Biomass + Liquid substrate formation

(Acid, COD and BOD)

$K' \downarrow$ Methanogenic microbial action

Biomass + Gaseous substrate formation

(Methane and Carbon dioxide)

The rate of degradation of substrates (COD) can be written as:

$$-\mathrm{d}s/\mathrm{d}t = K'S \qquad (1)$$

with:

$$-\mathrm{d}s/S = K'\mathrm{d}t,$$

it follows:

$$\ln S/S_0 = K't,$$

and finally:

$$S = S_0 \mathrm{e}^{-Kt} \qquad (2)$$

where, S = Substrate concentration at time t in g/liter, S_0 = Initial substrate concentration in g/liter and K' = Rate constant in days.

With the above Eq. (2), experimental and predicted values of COD were calculated and are tabulated in Table 2 and shown in Fig. 12. Using Eq. (2) the statistical analysis delivered values for correlation coefficient, rate constant K and standard deviation of error based on the change of COD values, which are shown in Table 3.

The non-linear first order equation was solved by exponential regression analysis. This experimental values of the COD were compared with the predicted ones. The deviation between experimental and predicted values is not larger than 10–14% in any case.

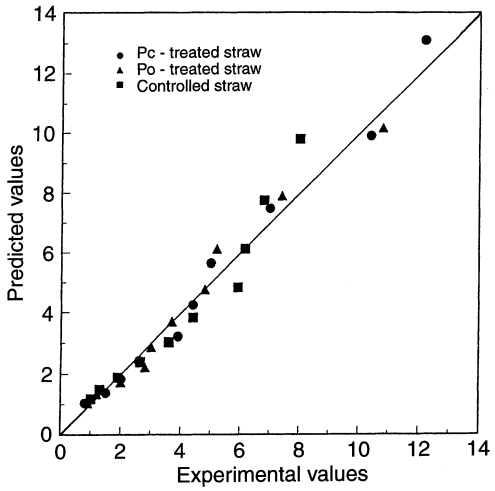

Fig. 12. Time courses of experimental and predicted values of COD

Table 2. Time courses of experimental and predicted values of COD

Sl No	Retention time (days)	P_C-treated straw		P_O-treated straw		Controlled straw	
		Exp	Pre	Exp	Pre	Exp	Pre
1	0	12.20	13.11	10.80	10.15	8.00	9.79
2	7	10.40	9.89	7.40	7.89	6.80	7.74
3	14	7.00	7.47	5.20	6.12	6.16	6.12
4	21	5.00	5.64	4.80	4.76	5.92	4.83
5	28	4.40	4.25	3.70	3.69	4.40	3.82
6	35	3.90	3.21	3.00	2.87	3.60	3.02
7	42	2.60	2.42	2.80	2.23	2.64	2.39
8	49	2.00	1.83	2.00	1.73	1.90	1.89
9	56	1.50	1.38	1.20	1.34	1.30	1.49
10	63	0.80	1.04	0.90	1.04	1.00	1.18

Exp = Experimental values
Pre = Predicted values

Table 3. Statistical analysis of COD values

Substrate	Correlation coefficient	*K*-Values day^{-1}	Standard deviation of error
P_C-treated straw	0.99	0.04	0.14
P_O-treated straw	0.98	0.04	0.13
Controlled straw	0.93	0.03	0.16

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