KEMENTERIAN PENDIDIKAN, KEBUDAYAAN,
RISET, DAN TEKNOLOGI
UNIVERSITAS DIPONEGORO
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT

Gedung ICT lantai 4
Jalan Prof. Sudarto, S.H.
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KONTRAK PELAKSANAAN PROGRAM PENELITIAN
PENELITIAN FUNDAMENTAL - REGULER
SUMBER DANA DIREKTORAT RISET, TEKNOLOGI, DAN PENGABDIAN KEPADA MASYARAKAT
DIREKTORAT JENDERAL PENDIDIKAN TINGGI, RISET, DAN TEKNOLOGI
KEMENTERIAN PENDIDIKAN, KEBUDAYAAN, RISET, DAN TEKNOLOGI
TAHUN ANGGARAN 2023

Nomor : 449A-22/UN7.D2/PP/VI/2023

Pada hari ini SELASA tanggal DUA PULUH bulan JUNI tahun DUA RIBU DUA PULUH TIGA, kami yang bertandatangan di bawah ini:

1. Prof. Dr. Jamari, S.T., M.T.	:	Ketua Lembaga Penelitian dan Pengabdian kepada Masyarakat Universitas Diponegoro berkedudukan di Kota Semarang, berdasarkan SK Rektor Universitas Diponegoro Nomor: 14/UN7.A/KP/XII/2022 tanggal 6 Desember 2022 tentang pengangkatan Ketua Lembaga Penelitian dan Pengabdian kepada Masyarakat Universitas Diponegoro periode masa jabatan 2022-2027, untuk selanjutnya disebut PIHAK KESATU;
2. Sutaryo, S.Pt., M.P., Ph.D.	:	Dosen Fakultas Peternakan dan Pertanian Universitas Diponegoro, dalam hal ini bertindak sebagai Ketua Pelaksana skema Penelitian Fundamental - Reguler Tahun Anggaran 2023 untuk selanjutnya disebut PIHAK KEDUA.

Berdasarkan Kontrak Pelaksanaan Program Bantuan Operasional Perguruan Tinggi Negeri Program Penelitian Tahun Anggaran 2023 antara Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi dengan Universitas Diponegoro Nomor: 121/E5/PG.02.00.PL/2023 tanggal 19 Juni 2023, disebutkan dalam pasal 5 ayat (4) bahwa Universitas Diponegoro mempunyai kewajiban membuat Kontrak Pelaksanaan Program Penelitian antara Ketua Lembaga yang membidangi penelitian dengan ketua pelaksana penelitian untuk pengaturan hak dan kewajiban pelaksanaan penelitian di lingkungan Universitas Diponegoro.

Maka PIHAK KESATU dan PIHAK KEDUA secara bersama-sama sepakat mengikatkan diri dalam suatu Kontrak Pelaksanaan Program Penelitian yang memuat: nama pelaksana; judul penelitian; ruang lingkup penelitian; sumber dana penelitian; nilai kontrak penelitian; tata cara dan tahapan pembayaran; jangka waktu pelaksanaan dan penyelesaian; hak dan kewajiban para pihak; batas akhir pelaporan; pencantuman pemberi dana penelitian dalam publikasi ilmiah; luaran penelitian; serah terima luaran penelitian; kesanggupan pelaksanaan penelitian; dan sanksi; dengan ketentuan dan syarat-syarat sebagaimana diatur dalam pasal-pasal sebagai berikut:

Pasal 1
Ruang Lingkup

PIHAK KESATU menugaskan kepada PIHAK KEDUA dan PIHAK KEDUA menerima penugasan dari PIHAK KESATU, untuk melaksanakan dan menyelesaikan program penelitian skema Penelitian Fundamental - Reguler tahun ke 1 dari rencana 2 tahun pada Tahun Anggaran 2023.

Pasal 2
Tim Peneliti, Judul, dan Dana Penelitian

(1) PIHAK KESATU menugaskan kepada PIHAK KEDUA untuk melaksanakan penelitian dengan Tim Peneliti dan Judul Penelitian sebagai berikut :

- Tim Peneliti : 1. Sutaryo, S.Pt., M.P., Ph.D.
  2. Prof. Ir. Agung Purnomoadi, M.Sc. Ph.D.
- Judul Penelitian : Eksplorasi Germinasi sebagai Metode Pre-Treatment untuk Meningkatkan Produksi Methan pada Biji Buah Pepaya dan Aplikasinya untuk Boosting Produksi Biogas dari Limbah Peternakan

(2) Dana pelaksanakan program penelitian dengan judul sebagaimana dimaksud pada ayat (1) adalah sebesar Rp. 62.500.000,- *(Enam puluh dua juta lima ratus ribu rupiah)* termasuk pajak;
(3) Dana sebagaimana dimaksud pada ayat (2) untuk selanjutnya disebut sebagai Dana Penelitian;
(4) Dana Penelitian dibebankan pada Daftar Isian Pelaksanaan Anggaran (DIPA) Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi Tahun Anggaran 2023, Nomor SP DIPA-023.17.1.690523/2023 revisi ke-4 tanggal 31 Maret 2023

Pasal 3
Tata Cara Pembayaran Dana Penelitian

(1) PIHAK KESATU akan membayarkan Dana Penelitian kepada PIHAK KEDUA secara bertahap dengan ketentuan sebagai berikut:
   a. Penyerahan dana tahap pertama sebesar 70% x Rp. 62.500.000,- = Rp. 43.750.000,- *(Empat puluh tiga juta tujuh ratus lima puluh ribu rupiah)*, yang akan dibayarkan oleh PIHAK KESATU setelah PIHAK KEDUA memenuhi persyaratan sebagai berikut:
      - Menandatangani kontrak pelaksanaan program penelitian;
      - Mengunggah revisi proposal sesuai dana yang disetujui dan surat pernyataan kesanggupan penyusunan laporan penelitian ke laman yang ditentukan oleh PIHAK KESATU;
      - Menyerahkan rincian anggaran belanja sebesar 100% sesuai dana yang disetujui disertai Pajak Penghasilan (PPh 21) dan Pajak Jasa (PPh 23)
   b. Penyerahan dana tahap kedua sebesar 30% x Rp. 62.500.000,- = Rp. 18.750.000,- *(Delapan belas juta tujuh ratus lima puluh ribu rupiah)*, dibayarkan oleh PIHAK KESATU kepada PIHAK KEDUA setelah PIHAK KEDUA mengunggah Laporan kemajuan/Antara penelitian dan Surat Pernyataan Tanggung Jawab Belanja (SPTB) ke laman yang ditentukan oleh PIHAK KESATU paling lambat tanggal 30 Agustus 2023 dan menyelesaikan seluruh kewajiban sesuai dengan ketentuan yang berlaku;
   c. Pembayaran Dana Penelitian tahap kedua sebesar 30% sebagaimana disebut pada ayat (1) butir b dilakukan dengan mentransferkan ke rekening PIHAK KESATU dalam posisi blokir.
(2) Dana Penelitian sebagaimana dimaksud pada ayat (1) tersebut akan dibayarkan melalui rekening atas nama PIHAK KEDUA pada bank yang ditunjuk oleh PIHAK KESATU;

Pasal 4
Pemblokiran Dana Penelitian

(1) PIHAK KEDUA memberikan kuasa penuh kepada PIHAK KESATU untuk melakukan blokir saldo sejumlah dana yang telah dibayarkan oleh PIHAK KESATU kepada PIHAK KEDUA apabila PIHAK KEDUA belum memenuhi segala kewajiban dan persyaratan pencairan;

(2) PIHAK KESATU tidak melakukan pemblokiran dana penelitian tahap pertama (70%) yang telah ditransferkan kepada PIHAK KEDUA;
(3) PIHAK KESATU melakukan pemblokiran dana penelitian tahap kedua (30%) yang telah ditransferkan kepada PIHAK KEDUA;
(4) Pembukaan blokir sebagaimana disebut pada ayat (3) dilakukan setelah PIHAK KEDUA menyelesaikan seluruh kewajibannya.

Pasal 5
Jangka Waktu Pelaksanaan Penelitian

Kontrak pelaksanaan Program penelitian ini berlaku mulai tanggal 19 Juni 2023 sampai dengan 10 Desember 2023.

Pasal 6
Monitoring dan Evaluasi

PIHAK KEDUA wajib mengikuti monitoring dan evaluasi penelitian yang dilaksanakan oleh PIHAK KESATU dengan persyaratan:
(1) Mengunggah Laporan Kemajuan Pelaksanaan Penelitian, catatan harian pelaksanaan penelitian dan Surat Pernyataan Tanggungjawab Belanja (SPTB) ke laman yang ditentukan oleh PIHAK KESATU;
(2) Mengumpulkan SPJ penggunaan dana tahap pertama (70%) sekurang-kurangnya dalam bentuk draf.

Pasal 7
Target Luaran

(1) PIHAK KEDUA berkewajiban untuk mencapai target luaran penelitian sebagaimana yang dijanjikan dalam proposal berdasarkan ketentuan yang berlaku;
(2) PIHAK KEDUA berkewajiban untuk melaporkan perkembangan pencapaian target luaran sebagaimana dimaksud pada ayat (1) kepada PIHAK KESATU.

Pasal 8
Hak dan Kewajiban Para Pihak

(1) PIHAK KESATU mempunyai hak:
   a. Menerima laporan kemajuan penelitian;
   b. Menerima laporan akhir tahun atau laporan akhir pelaksanaan penelitian;
   c. Melakukan pemantauan dan evaluasi;
   d. Menerima Surat Pernyataan Tanggungjawab Belanja (SPTB) atas dana penelitian yang telah ditetapkan;
   e. Melakukan pemantauan dan evaluasi kepada PIHAK KEDUA.
(2) PIHAK KEDUA mempunyai hak mendapatkan dana penelitian dari PIHAK KESATU
(3) PIHAK KESATU mempunyai kewajiban:
   a. Menyerahkan dana penelitian kepada PIHAK KEDUA;
   b. Melakukan pemantauan dan evaluasi;
   c. Melakukan penilaian luaran penelitian;
   d. Melakukan validasi luaran tambahan.
(4) PIHAK KEDUA mempunyai kewajiban:
   a. Mengunggah ke laman yang ditentukan oleh PIHAK KESATU atas dokumen sebagai berikut:
      1. Revisi proposal penelitian;
      2. Surat pernyataan kesanggupan pelaksanaan penelitian;
      3. Catatan harian pelaksanaan penelitian;
      4. Laporan kemajuan pelaksanaan penelitian;
      5. Surat Pernyataan Tanggungjawab Belanja (SPTB) atas dana penelitian yang telah ditetapkan;
      6. Laporan akhir tahun atau laporan akhir pelaksanaan penelitian;
      7. Menyelesaikan laporan pengunaan anggaran dana penelitian;
      8. Luaran penelitian;

b. PIHAK KEDUA wajib menyerahkan hasil penelitian kepada PIHAK KESATU melalui Berita Acara Serah Terima (BAST).
c. PIHAK KEDUA bertanggung jawab penuh atas pelaksanaan riset, pengadministrasian, pembelanjaan, dan pelaporan keuangan sebagaimana dimaksud pada ayat (1) sesuai dengan ketentuan yang berlaku;
d. Apabila dalam pelaksanaan penelitian terdapat sisa dana, maka PIHAK KEDUA wajib mengembalikan ke kas negara melalui PIHAK KESATU.
e. Apabila PIHAK KEDUA tidak dapat melaksanakan riset sebagaimana dimaksud pada ayat (1) maka PIHAK KEDUA wajib mengembalikan dana sebagaimana disebutkan pada ayat (2) ke kas negara melalui PIHAK KESATU.
f. Bilamana diperlukan, PIHAK KESATU dapat meminta kepada PIHAK KEDUA untuk menyerahkan dokumen hasil unggahan sebagaimana disebut pada ayat (2) dalam bentuk *hardcopy* dengan ketentuan sebagai berikut:
   a. Ditulis dalam kertas ukuran A4.
   b. Ditulis dengan *font Times New Romans* ukuran 12 spasi 1,5.
   c. Di sampul *(cover)* bagian bawah ditulis:

Di biayai oleh: DIPA Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi TA. 2023, SP DIPA-023.17.1.690523/2023 revisi ke-4 tanggal 31 Maret 2023 Nomor Kontrak Induk : 121/E5/PG.02.00.PL/2023 tanggal 19 Juni 2023 Nomor Kontrak Pelaksanaan Program: 449A-22/UN7.D2/PP/VI/2023 tanggal 20 Juni 2023

g. Menyerahkan laporan penggunaan dana penelitian (70% dan 30% dijilid menjadi satu) kepada PIHAK KESATU dalam bentuk *hardcopy (Soft Cover Laminating)* sebanyak 1 eksemplar asli dan 1 eksemplar disimpan PIHAK KEDUA paling lambat tanggal 10 Desember 2023.

Pasal 9
Penilaian Luaran

(1) Luaran penelitian dapat berupa luaran wajib dan luaran tambahan;
(2) Penilaian luaran penelitian dilakukan oleh Komite Penilai/*Reviewer* Luaran PIHAK KESATU sesuai dengan ketentuan yang berlaku.

Pasal 10
Perubahan Susunan Tim Pelaksana dan Substansi Pelaksanaan

(1) Perubahan terhadap susunan tim pelaksana dan substansi pelaksanaan Penelitian ini dapat dibenarkan apabila telah mendapat persetujuan tertulis dari Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi melalui PIHAK KESATU;
(2) Apabila PIHAK KEDUA selaku ketua pelaksana tidak dapat melaksanakan Penelitian ini, maka PIHAK KEDUA wajib mengusulkan pengganti ketua pelaksana yang merupakan salah satu anggota tim yang memenuhi persyaratan kepada PIHAK KESATU untuk diusulkan ke Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi.
(3) Dalam hal tidak terdapat pengganti ketua tim pelaksana penelitian sesuai dengan syarat dan ketentuan dalam panduan penelitian, maka penelitian dibatalkan dan dana dikembalikan ke Kas Negara.

Pasal 11
Pajak dan Meterai

(1) PIHAK KEDUA berkewajiban membayar pajak sesuai dengan ketentuan peraturan perundang-undangan di bidang perpajakan yang berlaku;

(2) Tata cara pembayaran pajak diatur oleh PIHAK KESATU dalam panduan pertanggungjawaban Keuangan Penelitian;
(3) PIHAK KEDUA berkewajiban memungut dan menyetor pajak ke kantor pelayanan pajak setempat yang berkenaan dengan kewajiban pajak sesuai dengan ketentuan peraturan perundang-undangan;
(4) Biaya Meterai dalam kontrak ini dibebankan kepada PIHAK KEDUA.

Pasal 12
Kekayaan Intelektual dan Aset Tetap yang dihasilkan

(1) Hak Kekayaan Intelektual yang dihasilkan dari pelaksanaan penelitian ini adalah milik negara yang dikelola dan diatur oleh Universitas Diponegoro sesuai dengan peraturan dan perundang-undangan;
(2) Setiap publikasi, makalah, dan/atau ekspos dalam bentuk apapun yang berkaitan dengan hasil penelitian ini wajib mencantumkan nomor Kontrak Pelaksanaan Program Penelitian dan pemberi dana dalam hal ini Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi dan Universitas Diponegoro sebagai pengelola pelaksanaan kegiatan penelitian;
(3) Bilamana pelaksanaan penelitian ini menghasilkan aset tetap maka PIHAK KEDUA berkewajiban menyerahkan kepada PIHAK KESATU yang dilampiri berita acara serah terima dengan ketentuan sebagai berikut:
    - Asset Tetap tersebut telah terdaftar dalam registrasi pengelolaan Barang Milik Negara;
    - Asset Tetap tersebut dilampiri dengan Standar Operasional Prosedure (SOP).
(4) Bilamana pelaksanaan penelitian ini menghasilkan aset tetap maka PIHAK KEDUA berkewajiban menyerahkan kepada PIHAK KESATU yang dilampiri berita acara serah terima dengan dilengkapi nomor Regitrasi Pengelolaan Barang Milik Negara;
(5) Hasil penelitian berupa aset tetap dari kegiatan ini dicatat secara tertib dan akuntabel dalam inventaris fakultas homebase ketua peneliti dan menjadi aset Universitas Diponegoro.

Pasal 13
Sanksi

Apabila sampai dengan batas waktu yang telah ditetapkan untuk melaksanakan pelaksanaan program penelitian telah berakhir, PIHAK KEDUA tidak melaksanakan kewajiban yang tercantum dalam Kontrak Pelaksanaan Program Penelitian ini, maka PIHAK KEDUA dikenai sanksi administratif sesuai dengan ketentuan peraturan perundang-undangan.

Pasal 14
Pembatalan Kontrak

Apabila dikemudian hari terhadap judul Penelitian sebagaimana dimaksud dalam Pasal 1 ditemukan adanya duplikasi dengan Penelitian lain dan/atau ditemukan adanya ketidakjujuran, itikad tidak baik, dan/atau perbuatan yang tidak sesuai dengan kaidah ilmiah dari atau dilakukan oleh PIHAK KEDUA, maka Kontrak Pelaksanaan Program Penelitian ini dinyatakan batal dan PIHAK KEDUA wajib mengembalikan dana yang telah diterima dari PIHAK KESATU.

Pasal 15
Keadaan Memaksa *(Force Majeure)*

(1) PARA PIHAK dibebaskan dari tanggungjawab atas keterlambatan atau kegagalan dalam memenuhi kewajiban yang dimaksud dalam Kontrak Pelaksanaan Program Penelitian yang disebabkan atau diakibatkan oleh peristiwa diluar kekuasaan PARA PIHAK yang dapat digolongkan sebagai keadaan memaksa *(force majeure)*;
(2) Peristiwa atau kejadian yang dapat digolongkan keadaan memaksa *(force majeure)* dalam Kontrak Pelaksanaan Program Penelitian ini antara lain: bencana alam, wabah penyakit, kebakaran, perang, blokade, peledakan, sabotase, revolusi, pemberontakan, huru-hara, serta adanya tindakan pemerintah dalam bidang ekonomi dan moneter yang secara nyata berpengaruh terhadap Kontrak Pelaksanaan Program Penelitian ini;

(3) Apabila terjadi keadaan memaksa *(force majeure)* maka pihak yang mengalami wajib memberitahukan kepada pihak lainnya secara tertulis, selambat-lambatnya dalam waktu 7 (tujuh) hari kerja sejak terjadinya keadaan memaksa *(force majeure)*, disertai bukti-bukti yang sah dari pihak yang berwajib, dan PARA PIHAK dengan itikad baik akan segera membicarakan penyelesaiannya.

Pasal 16
Penyelesaian Sengketa

Apabila terjadi perselisihan antara PIHAK KESATU dan PIHAK KEDUA dalam Kontrak Pelaksanaan Penelitian ini akan dilakukan penyelesaian secara musyawarah dan mufakat, sekiranya tidak tercapai penyelesaian secara musyawarah dan mufakat maka penyelesaian dilakukan melalui proses hukum dengan memilih tempat di Pengadilan Negeri Semarang, sebagai upaya hukum tingkat pertama dan terakhir.

Pasal 17
Adendum, Penutup dan Lain-lain

(1) PIHAK KEDUA menjamin bahwa penelitian dengan judul tersebut di atas belum pernah dibiayai dan/atau diikutsertakan pada Pendanaan Penelitian lainnya, baik yang diselenggarakan oleh instansi, lembaga, perusahaan atau yayasan, baik di dalam maupun di luar negeri;
(2) Hal-hal yang belum diatur dalam Kontrak Pelaksanaan Program Penelitian ini diatur kemudian antara PIHAK KESATU dan PIHAK KEDUA yang akan dituangkan dalam bentuk adendum dan merupakan bagian tak terpisahkan dari Kontrak Pelaksanaan Program Penelitian ini;
(3) Kontrak Pelaksanaan Program Penelitian ini dibuat rangkap 2 (dua), bermeterai cukup sesuai dengan ketentuan yang berlaku.

PIHAK KEDUA	PIHAK KESATU
Sutaryo, S.Pt., M.P., Ph.D.	Prof. Dr. Jamari, S.T., M.T.
NIP. 197501312002121002	NIP. 197403042000121001

LAPORAN AKHIR

PENELITIAN FUNDAMENTAL – REGULER
SUMBER DANA DRPM KEMENDIKBUD, DAN RISTEK
TAHUN ANGGARAN 2023

Eksplorasi Germinasi sebagai Metode Pre-Treatment untuk Meingkatkan Produksi Methan pada Biji Buah Pepaya dan Aplikasinya untuk Boosting Produksi Biogas dari Limbah Peternakan

SUTARYO, S.Pt., M.P., Ph.D

Prof. Dr. Ir. Agung Purnomoadi, M.Sc

Dibiayai oleh:
DIPA Direktorat Riset, Teknologi, dan Pengabdian Kepada Masyarakat,
Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi
Kementrian Pendidikan, Kebudayaan, Riset, dan Teknologi TA. 2023
Nomor Kontrak Induk : 121/E5/PG.02.00.PL/2023 tanggal 19 Juni 2023
No Kontrak Pelaksanaan Program: 449A-22/UN7.D2/PP/VI/2023 tanggal 20 Juni 2023

UNIVERSITAS DIPONEGORO

TAHUN 2023

HALAMAN PENGESAHAN LAPORAN AKHIR
PENELITIAN FUNDAMENTAL - REGULER

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1. Judul Penelitian	: Explorasi Germinasi sebagai Metode Pre-treatment untuk Meningkatkan Produksi Methan pada Biji Buah Pepaya dan Aplikasinya untuk Boosting Produksi Biogas dari Limbah Peternakan
2. Ketua Peneliti	
a. Nama Lengkap	: Sutaryo, S.Pt., M.P., Ph.D
b. Jenis Kelamin	: Laki-Laki
c. Gol/Pangkat/NIP	: IVa / Pembina / 1975013120021210002
d. Jabatan Fungsional	: Lektor Kepala
e. Jabatan Struktural	: Kaprodi S3 Peternakan
f. Fakultas/Jurusan	: Peternakan dan Pertanian/Peternakan
g. Pusat Penelitian	: Laboratorium Produksi Ternak Potong dan Perah
h. Alamat Kantor	: Jalan Prof. Jacub Rais, Kampus Universitas Diponegoro, Tembalang, Semarang, Kode Pos 50275, Tel/Faks: (024) 7474750, Pos-el:fpp@undip.ac.id
i. Alamat Rumah	: Jl Dinar Mas Utara V No.1 Meteseh-Tembalang
3. Perguruan Tinggi	: Universitas Diponegoro Semarang
4. Jangka Waktu Penelitian Penelitian	: 7 bulan
a. Biaya yang diperlukan	: Rp 62.500.000,-
b. Biaya dari Instansi	: Tidak ada
Jumlah	: Rp 62.500.000,- (Enam puluh dua juta lima ratus ribu rupiah)

Semarang, 2 Desember 2023

Mengetahui,
Wakil Dekan II FPP

Prof. Agus Setiadi, S.Pt., M.Si., Ph.D

NIP. 197708052002121003

Ketua Peneliti

Sutaryo, SPt., M.P., Ph.D

NIP. 1975013120021210002

Menyetujui
Ketua LPPM UNDIP

Prof. Dr. Jamari, S.T., M.T.
NIP. 197403042000121001.

Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. **HASIL PELAKSANAAN PENELITIAN:** Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian meliputi data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

Tujuan dari penelitian ini yaitu mengetahui produksi metan dari penggunaan *co-substrate manure* sapi perah dan biji pepaya non germinasi dan digerminasi. Penelitian dilakukan secara eksperimental menggunakan 4 perlakuan yaitu T0 (kontrol 100% *manure* sapi perah); T1 (90% *manure* sapi perah dan 10 % biji pepaya non germinasi); T2 (90% *manure* sapi perah dan 10% biji pepaya digerminasi); dan T3 (80% *manure* sapi perah dan 20% biji pepaya digerminasi). Penelitian ini dilaksanakan selama 66 hari atau 3 *hydraulic retention time* (HRT) dengan menggunakan digester kontinyu. Penelitian ini juga mengevaluasi produksi methan dari biji pepaya germinasi dan non germinasi pada berbagai kondisi fisik biji papaya (segar dan kering) menggunakan digester tipe *batch.* Sampai pada saat laporan akhir ini disusun untuk tahap evaluasi produksi dari biji papaya sebagai substrat tunggal menggunakan digester type batch masih berlangsung, sedangkan untuk tahap evaluasi co-digesti manure sapi perah dan biji papaya menggunakan digester kontinyu sudah selesai dilaksanakan.

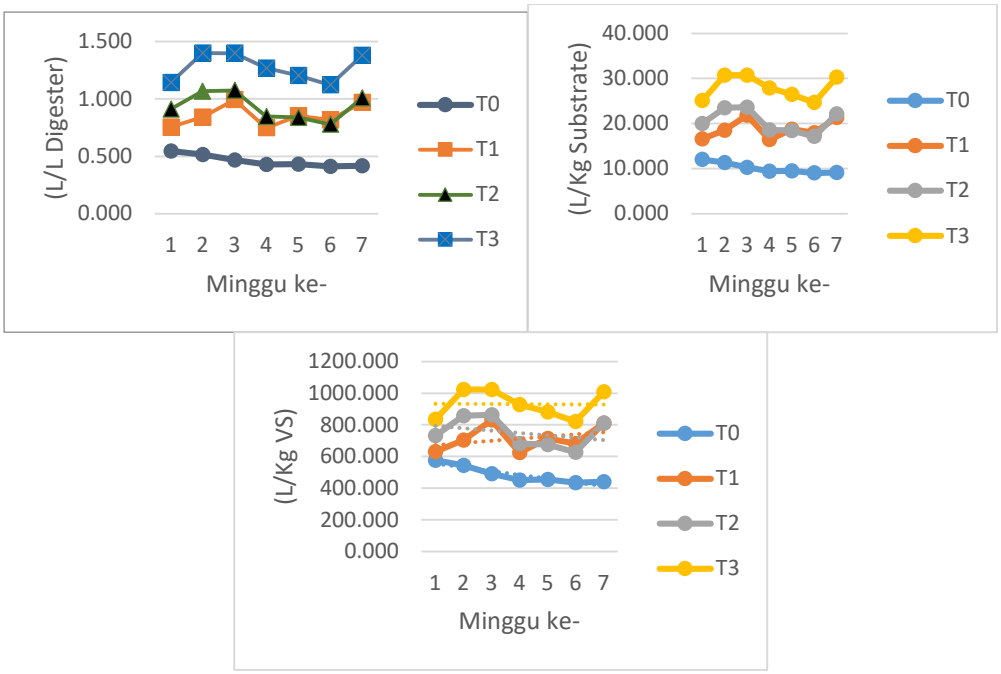
A. Produksi Metan

Hasil produksi metan dari penggunaan *co-substrate manure* sapi perah dan biji pepaya non germinasi dan digerminasi ditampilkan pada Tabel 1, sedangkan grafik produksi metan ditampilkan pada Gambar 1.

Tabel 1. Methane yield, TAN concentration, VFA total, VS reduction and pH digester

Treatments	Methane Production			TAN (mg/L)	VFA total (mM)	VS reduction (%)	pH
	L/L digester volume	L/Kg Substrate	L/Kg VS added				
T0	0.46 ± 0.09^{a}	10.19 ± 1.94^{a}	487.95 ± 93.12^{a}	147.28 ± 65.07^{a}	43.99 ± 32.79^{a}	28.33 ± 11.49	7.37 ± 0.20
T1	0.86 ± 0.19^{b}	18.91 ± 4.08^{b}	718.34 ± 155.16^{b}	265.72 ± 81.51^{b}	4.45 ± 3.12^{b}	35.75 ± 12.26	7.43 ± 0.32
T2	0.94 ± 0.21^{c}	20.62 ± 4.72^{c}	753.60 ± 172.37^{b}	263.27 ± 62.07^{b}	2.33 ± 2.50^{b}	38.41 ± 13.60	7.40 ± 0.37
T3	1.27 ± 0.32^{d}	28.02 ± 7.07^{d}	932.69 ± 235.24^{c}	393.37 ± 139.72^{c}	2.06 ± 1.73^{b}	33.41 ± 17.21	7.47 ± 0.40
Sign	*	*	*	*	*	ns	ns

T0 (kontrol 100% *manure* sapi perah); T1 (90% *manure* sapi perah dan 10 % biji pepaya non germinasi); T2 (90% *manure* sapi perah dan 10% biji pepaya digerminasi); T3 (80% *manure* sapi perah dan 20% biji pepaya digerminasi); * significant; ^{ns} non-significant, ^{abcd} *Means in the same row without common letters are different at $p<0.05$.*



Gambar 1. Grafik Produksi Metan L/L digester, L/Kg Substrate, dan L /Kg VS

Berdasarkan hasil analisis statistik diketahui bahwa penggunaan *co-substrate manure* sapi perah dan biji pepaya non germinasi dan digerminasi berpengaruh nyata (P<0,05) terhadap produksi metan. Masing-masing perlakuan memberikan respon yang berbeda, dengan produksi metan tertinggi terdapat pada perlakuan T3 dibandingkan kontrol atau perlakuan lainnya pada masing-masing satuan produksi metan (Gambar 1). Diduga, tingginya produksi metan pada T3 disebabkan oleh tingginya kandungan protein yang terkandung pada biji pepaya digerminasi. Selain itu proses germinasi dapat mengubah senyawa kompleks protein menjadi asam amino sehingga mempercepat tahap hidrolisis, asam amino dapat langsung dimanfaatkan oleh mikroorganisme fermentatasi untuk memproduksi VFA, etanol, laktat, CO_2, dan H_2.

Hal tersebut sesuai dengan pernyataan Ferdiawan *et al*. (2019) yaitu proses germinasi adalah salah satu tahapan pertumbuhan pada biji yang dapat meningkatkan nilai gizi khususnya protein. Biji mengandung asam amino yang berikatan satu dengan yang lain membentuk makromolekul protein sehingga sulit dimanfaatkan. Selama proses germinasi, terjadi peningkatan aktivitas degradasi protein sebagai cadangan dalam biji oleh enzim protease endogen dan membentuk peptida pendek maupun asam amino bebas yang dibutuhkan selama proses pertumbuhan biji. Hal ini berakibat terjadinya peningkatan kadar peptida dan asam amino bebas, dijelaskan lebih lanjut bahwa pada biji digerminasi terbukti mampu menurunkan aktivitas trypsin inhibitor dan berpengaruh terhadap profil asam amino bebas kecambah. Asam amino bebas yang terkonfirmasi pada biji digerminasi jumlahnya lebih tinggi sehingga dapat digunakan oleh mikroorganisme dalam memproduksi metan (Kanetro, 2009; Yoshari *et al.,* 2023).

B. Variabel di Dalam Digester

Penggunaan *co-substrate manure* sapi perah dan biji pepaya non germinasi dan digerminasi berpengaruh nyata (P<0,05) terhadap variabel TAN. Berdasarkan uji lanjut, perlakuan T3 mempunyai konsentrasi tertinggi dibandingkan kontrol dan perlakuan lain. Konsentrasi TAN berasal dari sisa metabolisme, bahan organik, sisa pakan, dan bangkai organisme maupun mikroorganisme. Konsentrasi TAN yang baik untuk biogas yaitu 200-1500 mg/l dan jika kandungannya melebihi 3.000 mg/l dapat menghambat proses fermentasi bahkan bersifat racun (Waskito, 2011; Putra *et al*., 2019).

Penggunaan *co-substrate manure* sapi perah dan biji pepaya non germinasi dan digerminasi berpengaruh nyata (P<0,05) terhadap variabel VFA. Berdasarkan uji lanjut, perlakuan T1, T2, dan T3 mempunyai konsentrasi tertinggi dibandingkan kontrol. Pemberian biji pepaya dapat meningkatkan konsentrasi VFA, kandungan makronutrien yang terkandung dalam biji pepaya dimanfaatkan oleh bakteri fermentasi untuk membentuk VFA. VFA merupakan salah satu variabel penting dalam produksi biogas. Konsentrasi VFA dapat dijadikan indikator baik atau tidaknya proses asidogenesis. Proses asidogenesis merupakan pencernaan gula sederhana, asam amino, asam lemak rantai panjang dan gliserol menjadi VFA (asam asetat, asam propionat, asam butirat), H_2, dan CO_2 (Akintunde *et al*., 2022).

Penggunaan *co-substrate manure* sapi perah dan biji pepaya non germinasi dan digerminasi tidak berpengaruh nyata (P>0,05) terhadap persentase RBO. Hal tersebut disebabkan oleh kemampuan bakteri pada setiap perlakuan tidak berbeda jauh dalam mencerna bahan organik. Bahan organik digunakan oleh bakteri anaerob sebagai sumber makanan (Darwin *et al*., 2016), sedangkan RBO menunjukkan kemampuan bakteri anaerob dalam merombak bahan organik untuk memproduksi metan. Semakin tinggi RBO memberikan arti bahwa bahan organik yang tereduksi dalam isian biogas masih melimpah sehingga masih berpotensi dalam memproduksi metan (Istiadi *et al*., 2020).

Penggunaan *co-substrate manure* sapi perah dan biji pepaya non germinasi dan digerminasi tidak berpengaruh nyata (P>0,05) terhadap nilai pH. Walaupun tidak berpengaruh nyata, namun pH yang dihasilkan masih dalam kategori normal (7.37-7.47) sehingga tidak mengganggu aktivitas bakteri penghasil metan. Dijelakan oleh Haryanto *et al*., (2021) produksi biogas melalui proses degradasi anaerobik dapat berjalan baik pada pH sekitar 5,5-8,5. Nilai pH optimal untuk bakteri asidogenik berkisar 5,2-6,3, sedangkan untuk bakteri metanogenik adalah 6,7-7,5 (Deubelin dan Steinhauser, 2008), jika pH tidak sesuai maka bakteri tidak dapat hidup sehingga berpengaruh terhadap produksi biogas (Haryanto *et al*., 2021).

D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta mengunggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui BIMA.

Evaluasi produksi methane dari biji pepaya sebagai substrate tunggal, saat ini masih berlangsung dengan demikian penulis belum bisa menyusun draft publikasi secara utuh. Namun demikian, apabila penelitian tersebut sudah selesai dilaksanakan akan segera disusun menjadi satu draft publikasi yang kompehensif sehingga, mempunyai informasi yang menyeluruh dan komprehensif dan mempunyai kebaharuan yang tinggi yang memudahkan dalam publikasi pada suatu jurnal internasional bereputasi.

E**. PERAN MITRA:** Tuliskan realisasi kerjasama dan kontribusi Mitra baik *in-kind* maupun *in-cash* (untuk Penelitian Terapan, Penelitian Pengembangan, PTUPT, PPUPT serta KRUPT). Bukti pendukung realisasi kerjasama dan realisasi kontribusi mitra dilaporkan sesuai dengan kondisi yang sebenarnya. Bukti dokumen realisasi kerjasama dengan Mitra diunggah melalui BIMA.

Penelitian ini tidak melibatkan mitra.

F. **KENDALA PELAKSANAAN PENELITIAN**: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Terdapat dua rangkaian penelitian pada tahun pertama, yaitu: evaluasi kinerja digester biogas type kontinyu dengan substrat campuran antara biji pepaya germnasi dan non germinasi dan produksi methane dari biji pepaya germinasi dan non germinasi sebagai substrate tunggal menggunakan digester type batch. Masih masih penelitian membutuhkan waktu tiga bulan sehingga penelitian secera keseluruhan membutuhkan waktu efektif enam bulan. Sampai laporan akhir ini disusun, tahap evaluasi produksi methane dari digester type batch masih berlangsung. Sebenarnya keduanya bisa dilaksanakan secara sinergis, namun demikian karena keterbatasan tenaga peneliti dan agar pelaksanaan penelitian bisa berjalan secara optima akhirnya dilaksanakan secara bertahap.

G**. RENCANA TAHAPAN SELANJUTNYA:** Tuliskan dan uraikan rencana penelitian di tahun berikutnya berdasarkan indikator luaran yang telah dicapai, rencana realisasi luaran wajib yang dijanjikan dan tambahan (jika ada) di tahun berikutnya serta *roadmap* penelitian keseluruhan. Pada bagian ini diperbolehkan untuk melengkapi penjelasan dari setiap tahapan dalam metoda yang akan direncanakan termasuk jadwal berkaitan dengan strategi untuk mencapai luaran seperti yang telah dijanjikan dalam proposal. Jika diperlukan, penjelasan dapat juga dilengkapi dengan gambar, tabel, diagram, serta pustaka yang relevan. Pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai.

Menyelesaikan penelitian evaluasi produksi methane dari biji pepaya sebagai substrat tunggal dengan menggunakan digester type batch. Segera setelah penelitian selesai maka data segera ditabulasi dan dianalisis secara statistik dan segera disusun draft publikasi dan disubmit pada jurnal internasional bereputasi.

H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan akhir yang dicantumkan dalam Daftar Pustaka.

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Case Report

Evaluation of germination as pretreatment method to increase methane production: A case study in papaya seed

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ABSTRACT

This research aims to evaluate the effect germination of papaya seeds (GPS) on methane production (batch mode) and the application of GPS in co-digestion (continuous mode). Batch mode study showed that, compare to non-germinated (NGPS), GPS can increase methane production ($p < 0.05$) by 180 %, 57 %, and 81 % for fresh GPS, fresh GPS meal, and dry GPS, respectively. Methane yields of continuous study were 173.58, 260.71; 269.92; and 329.35 L/kg volatile solid for control; 90 % dairy cow manure (DCM) and 10 % NGPS; 90 % DCM and 10 % GPS; 80 % DCM and 20 % GPS. Germination can increase ($p < 0.05$) methane production of PS.

1. Introduction

In the livestock sector, efforts made to decrease greenhouse gas emissions and create renewable energy are by utilizing livestock manure for biogas production [1]. Biogas production process is influenced by several factors, one of which is the substrate condition [2] where this will determine the quality and quantity of the resulting biogas [3]. Dairy cow manure (DCM) has the potential to be processed anaerobically for biogas production either as a single substrate (mono-substrate) or a co-substrate with other organic materials [2,4]. Co-substrate is an effort to increase methane production [5] by meeting the nutritional needs of microorganisms thereby increasing methane production by up to 60–75 % [6]. In making co-substrate, DCM can be mixed using various types of organic materials available in nature, including agricultural waste [4].

In 2022, the total production of fruit crops in Indonesia reaches 27712182 tonnes/year. One of the main commodities is papaya (*Carica papaya* L.). The Central Statistics Agency (Indonesia) stated that the production of papaya fruits in Indonesia reaches 1,089,578 tons [7], with an average per capita consumption in Indonesia of 36.85 g/week [8]. The high production and consumption of papaya fruit is in line with the large amount of waste. One of the wastes is papaya seeds (PS). The PS has a proportion of 16 % of the weight of papaya fruit [9] so the PS waste in Indonesia in 2022 can reach 174,332.5 tonnes. People use PS for nursery purposes, but with certain criteria, seeds that do not meet the nursery criteria are considered waste. However, they still have the potential to be utilized, because they contain high levels of nutrients [10]. The PS contains 27.41 % crude protein, 28.61 % crude fat, and 19.70 % carbohydrates [11]. Food processing technology always progresses from time to time. Therefore, it does not rule out the possibility that papaya fruit will be processing to be ready to eat food in industrial scale in the future. Hence, it will make easier to handle the waste produced, for instance as animal feed, processed anaerobically for biogas production, or through composting.

Research related to the use of PS as a co-substrate with DCM to increase methane production is still limited. Previous research studies the combination of cow manure and papaya waste including fermented PS and peels as biogas substrates. This co-digestion can increase methane production by 50 % [12]. Efforts to increase methane production using fruit seed waste, especially PS waste, have not been widely explored. Before using as a co-substrate with DCM, the PS waste can be pretreated using a germination process. Germination is an effort to optimize the nutrient content, especially proteins [13].

The proteins in PS contain amino acids that bond with each other. During the germination process, there is an increase in protein degradation activity in the seeds by endogenous protease enzymes, thereby forming short peptides and free amino acids needed during the seed growth process [13]. In utilizing germinated seeds as a biogas substrate, the amino acids contained in the germinated seeds are thought to be able to speed up the hydrolysis stage so they can be utilized by fermentation microorganisms. This research aims to evaluate the effect of germination

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pretreatment on the methane production from PS which was evaluated using batch-type digesters and continuous stirred tank reactor (CTSR) digesters. In continuous experiments, the PS was utilized as a co-substrate with DCM.

2. Materials and methods

2.1. Experimental set up

This research consisted of two sets of research, namely batch mode experiment and continuous mode experiment. The first research aimed to evaluate the effect of germination pretreatment on methane production from PS using batch-type digesters. The second research aimed to apply PS that had been pretreated as a co-substrate with DCM using CTSR-type digesters. In batch research, there were 8 treatments, namely fresh non-germinated PS (FNGPS), fresh non-germinated PS meal (FNGPSM), fresh germinated PS (FGPS), fresh germinated PS meal (FGPSM), dry non-germinated PS (DNGPS), dry non-germinated PS meal (DNGPSM), dry germinated PS (DGPS), and dry germinated PS meal (DGPSM). In continuous research, there were 4 treatments, namely T0 (100 % DCM), T1 (90 % DCM and 10 % FNGPSM), T2 (90 % DCM and 10 % FGPSM), and T3 (80 % DCM and 20 % FGPSM).

2.2. Batch experiment

Batch experiments were carried out using 500 mL glass bottles with a method developed by Ref. [14]. The starter as much as 200 mL was added into the digester, and then PS was added according to the treatments. For fresh PS either without germination or with germination, as much as 10 g of PS was added. Meanwhile, for dry PS either without germination or with germination, as much as 4 g of PS was added. Those were conducted to reach the ratio of substrate to starter of around 1:1 (basis of VS). For the blank, the bottle was filled with 100 % starter. All batch digesters were closed with rubber plugs and clamped using aluminium crimps. After that, the flushing was conducted using nitrogen for 2 minutes. The batch digesters were then placed in an incubator at a temperature of 37 °C for 90 d. The net methane production was calculated by subtracting the methane production from the blank digester from methane production from the treatment digester. The batch mode research was carried out in triplicate.

2.3. Continuous experiment

Continuous experiments were carried out using four CTSR digesters with treatments T0, T1, T2, and T3. Each CSTR digester had a capacity of 7000 mL and an active volume of 75 % or 5250 mL. Stirring was carried out continuously using a propeller system with a rotation speed of 36 rpm. The initial stage (the adaptation stage) began with filling the active volume of the digester using a starter. In mesophilic conditions, the average hydraulic retention time (HRT) required to produce methane ranges from 15 to 30 d [15]. In this study, the adaptation stage was carried out in 1 HRT, namely for 22 d. During the adaptation period, the slurry was removed and the substrate was added in the same amount, namely 238.6 g. The substrate used in the adaptation period was manure made by diluting feces with water in a ratio of 1:1.6 (w/w) to adjust total solids (TS) in DCM [16]. After the adaptation stage, the data collection stage was carried out. This stage was conducted by removing the slurry and filling the mixed substrate of PS and DCM continuously every day with the same amount (238.6 g). The CTSR digesters were placed in an incubator at a temperature of 35–37 °C.

2.4. Starter and substrate

The starter in batch experiments was manure made by diluting dairy cow feces with water in a ratio of 1:1.6 (w/w). The feces were obtained from dairy cows during the lactation period in the Friesian Holstein (FH) Cowshed, Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia. Before use, the starter was stored in anaerobic conditions for ±2 weeks, then a filtering process was carried out to obtain the liquid fraction. The filtering process aims to get a uniform starter and minimize biogas production from the starter [17]. For continuous experiments, the starter used was digested slurry obtained from the active biogas digester at the Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia. It was directly transferred to the laboratory scale digesters. The characteristics of the starter for batch experiments were 1.44 ± 0.15 %, 0.90 ± 0.11 %, and 7.29 ± 0.02 for TS, VS, and pH values, respectively. Meanwhile, the characteristics of the starter for continuous experiments were 4.81 ± 0.26 %, 3.88 ± 0.13 %, and 7.54 ± 0.03 for TS, VS, and pH values, respectively.

The PS used as a co-substrate were obtained from cut fruit sellers at Meteseh Market, Semarang, Indonesia. The PS were collected and then the sarcotesta layer was removed. After that, they were washed and sorted. Without the germination pretreatment, there were four types of PS.

- The fresh non-germinated PS (FNGPS)
- The fresh non-germinated PS meal (FNGPSM) was the fresh seeds that were ground using a kitchen-mixer for 30 seconds.
- The dry non-germinated PS (DNGPS) were the fresh seeds that were dried in the sun.
- The dry non-germinated PS meal (DNGPSM) was the fresh seeds that were dried in the sun and ground using a kitchen-mixer for 30 seconds.

The germination process was carried out using a method developed by Ref. [18]. First, the fresh seeds were soaked in warm water with an initial temperature of 50 °C for 24 h. Then, they were rinsed using running water and drained. The seed germination process was carried out using a plastic bottle with a hole in the top and bottom and a planting medium in the form of cotton. The plastic bottle was then wrapped in black plastic and hung. To maintain humidity, the bottle was sprayed every two d. Harvesting of papaya seeds was conducted after 7–14 d of planting (sprout length 1–3 mm). With the germination pretreatment, there were four types of PS.

- The fresh germinated PS (FGPS).
- The fresh germinated PS meal (FGPSM) was the fresh germinated seeds that were ground using a kitchen-mixer for 30 seconds.
- The dry germinated PS (DGPS) were the fresh germinated seeds that were dried in the sun.
- The dry germinated PS meal (DGPSM) was the fresh germinated seeds that were dried in the sun and ground using a kitchen-mixer for 30 seconds.

In continuous experiments, the types of PS used were FNGPSM and FGPSM. The preparation of FNGPSM and FGPSM was explained in previous paragraphs. The use of FNGPSM and FGPSM as co-substrates with DCM was carried out to facilitate the feeding process in the CSTR digester, considering that the digester was used as a laboratory scale digester. Before use, PS were analysed. The results of the analysis of the nutrient content of PS are presented in Table 1. The PS were also analysed using the Scanning Electron Microscope energy dispersive X-ray (SEM-EDX). The SEM-EDX analysis results are presented in Fig. 1. Furthermore, the characteristics of mixed substrates in the continuous experiments are presented in Table 2.

2.5. Analytical methods

Methane production measurements in the batch experiments were carried out on d 3, 7, 12, 20, 30, 45, 60, 75, and 90 at 10.00 a.m. local time. The resulting methane production during batch experiments was expressed into standard state volume [19] with the temperature and

Table 1
Nutrient content of non-germinated and germinated papaya seeds.

Content (%)	Dry papaya seeds meal	
	Non-germination	Germination
Moisture	10.52	8.00
Total solid	89.48	92.00
Volatile solid	82.98	85.41
Ash content	6.50	6.59
Crude protein	27.29	28.72
Crude fat	28.62	20.58
Crude fibre	27.27	34.89
Carbohydrate	9.55	6.94
Neutral detergent fibre	55.86	57.11
Acid detergent fibre	47.19	48.13
Hemicellulose	8.67	8.98
Lignin	37.23	36.30
Cellulose	9.96	11.83

pressure according to IUPAC regulations, namely a temperature of 0 °C or 273 K and a pressure of 1 atm. Measurements of methane production in continuous experiments were carried out every d for 3 HRTs. Biogas generated from batch and continuous type digesters flowed into a 500 ml bottle containing 4 % NaOH solution (Merck®, Cat No. 1064981000) using a 5 mm diameter Teflon hose. The NaOH solution was replaced with a new solution once a week. The methane gas was then collected using a Tedlar gas bag (Hedetech-Dupont, China) with a volume of 1 and 10 L. Methane production was measured using the liquid displacement method in which the volume of water in the measuring tube was replaced by methane gas, as done by Ref. [20]. Measurement of total ammonia nitrogen (TAN) concentration was conducted using photometric kits (cat. no. 1.00683.0001) NOVA 60 A Spectroquant ®. Volatile fatty acid (VFA) concentration was measured using Gas Chromatography-Mass Spectrometry (Bruker SCION 436-GC) and pH measurement was carried out using a pH meter (Ohaus ® ST300). Measurements of the ammonia nitrogen (TAN), volatile fatty acids (VFAs), VS reduction, and pH values of digested slurries in continuous research were carried out once a week.

TS analysis was carried out in an oven at 105 °C for 7 h. Ash analysis was carried out by ashing at a temperature of 550 °C for 7 h. VS was calculated using the difference between TS and ash contents [21]. Crude fat and protein contents were analysed using the Soxhlet extraction method and the Kjeldahl method, respectively. The nitrogen (N) value was obtained from the protein value divided by 6.25. The organic carbon value and C/N ratio were calculated using a method reported by Ref. [22]. Crude fibre and carbohydrates were analysed using the gravimetric method and the volumetric method, respectively. Acid detergent fibre (ADF) was analysed using AOAC and Neutral detergent fibre (NDF) was analysed using the VanSoest method. Hemicellulose was calculated by subtracting the NDF and ADF values [23]. Cellulose and lignin analysis were determined using the gravimetric method [24]. Data were analysed using Analysis of Variance (ANOVA) with a significance level of 5 % if the results were significantly different ($p < 0.05$) then continued using Duncan's Multiple Range Test (DMRT) analysis [25].

3. Results and discussion

3.1. Batch experiment

Methane production from various treatments is presented in Table 3 and Fig. 2, while the percentage increase in methane production due to the germination and grinding pretreatments is presented in Table 4. The germination and grinding pretreatments had a significant effect ($p < 0.05$) on methane production on d 30, 60, and 90.

The highest methane production was produced from FGPSM which was fresh germinated PS meal. FGPS contained the main nutrients in the form of proteins which can be utilized by bacteria to generate methane (Table 2). In the germination process, there is an increase in protein degradation activity in the seeds by endogenous protease enzymes,

Table 2
Characteristics of mixed substrates in the co-digestion process using continuous digesters.

Treatments	TS (%)	VS (%)	Crude protein (%)	VS proportion of PS in the mixed substrate (%)	pH	C/N ratio
T0	6.74	5.95	1.48	0	7.40	21,73
T1	8.19	7.34	2.04	70.96	6.98	18,98
T2	8.08	7.24	2.26	70.36	6.80	18,14
T3	9.25	8.45	2.36	84.23	6.54	16,70

Table 3
Cumulative methane yield in batch experiments.

Treatments	Cumulative methane yield (L/kg VS)		
	Bo 30	*Bo* 60	*Bo* 90
FNGPS	89.50 ± 17.90^{a}	133.78 ± 21.12^{a}	159.22 ± 22.97^{a}
FNGPSM	310.91 ± 21.48^{ef}	338.70 ± 33.69^{bc}	351.44 ± 35.14^{b}
FGPS	275.90 ± 29.29^{de}	399.77 ± 24.21^{c}	446.02 ± 19.60^{d}
FGPSM	379.93 ± 45.73^{f}	497.05 ± 48.64^{d}	551.97 ± 56.39^{e}
DNGPS	121.00 ± 5.64^{ab}	156.32 ± 7.68^{a}	184.47 ± 6.54^{a}
DNGPSM	278.20 ± 54.18^{de}	388.18 ± 87.52^{c}	398.15 ± 89.01^{bc}
DGPS	173.67 ± 33.77^{bc}	258.50 ± 33.13^{b}	333.06 ± 35.38^{b}
DGPSM	213.70 ± 125.04^{cd}	393.71 ± 119.04^{c}	430.85 ± 111.61^{cd}

[abcd] Means in the same column without common letters are different at $p < 0.05$.

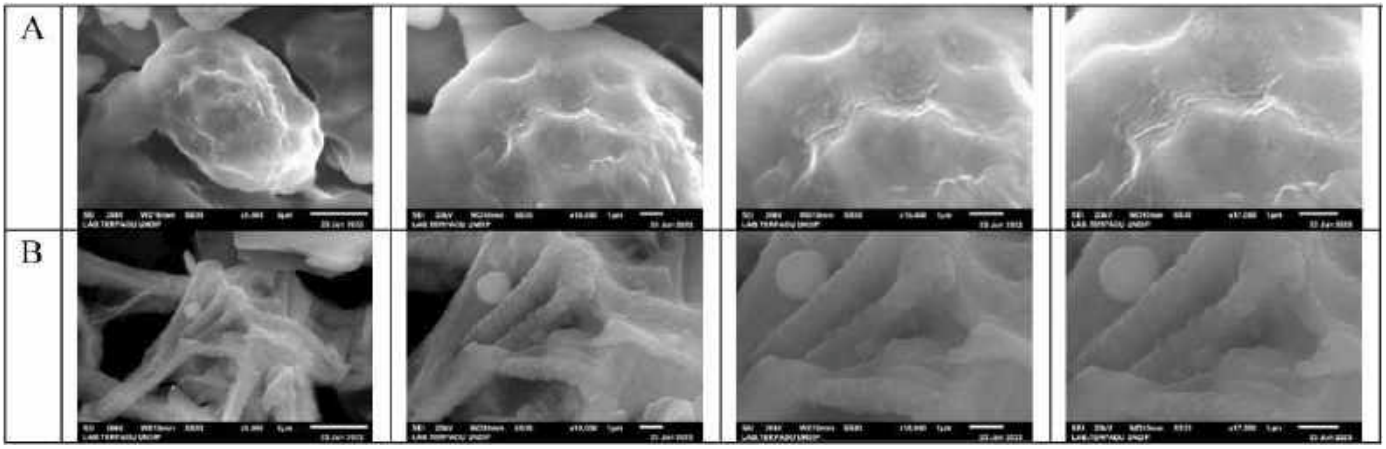

Fig. 1. SEM analysis results with magnification 5000 × , 10,000 × , 15,000 × , and 17,000 × (A = fresh non germinated PS; B = fresh germinated PS).

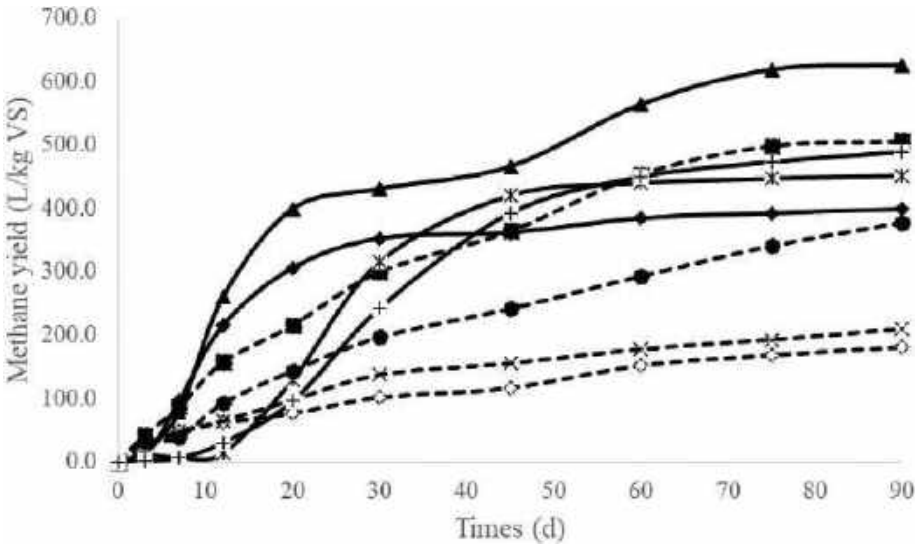

Fig. 2. The ultimate methane yield of the batch experiment (◇: fresh non-germinated PS (FNGPS); ◆: fresh non-germinated PS meal (FNGPSM); ■: fresh germinated PS (FGPS); ▲: fresh germinated PS meal (FGPSM); x: dry non-germinated PS (DNGPS); Ж: dry non-germinated PS meal (DNGPSM); ●: dry germinated PS (DGPS); +: dry germinated PS meal (DGPSM).

Table 4
Percentage increase in methane production from germination and grinding pretreatments.

Treatments	Increase in production of methane (%)
FNGPSM vs. FNGPS	120.75
FGPSM vs. FGPS	23.77
FGPS vs. FNGPSM	180.50
FGPSM vs. FNGPSM	57.26
DNGPSM vs. DNGPS	116.30
DGPS vs. DNGPS	80.98
DGPSM vs. DGPS	29.13
DGPSM vs. DNGPSM	8.04

resulting in short peptides and free amino acids needed during the seed growth process [13]. Thus, the FGPS will be more easily utilized by anaerobic microorganisms in the digester than FNGPS. The grinding pretreatment also affected methane production. The grinding process was a physical pretreatment process to reduce the particle size and increase the surface area of PS so the available organic materials could be more easily utilized by anaerobic microorganisms. This was in line with research conducted by Ref. [26] that the methane content will increase with reducing the size of the substrate particles. Additionally [27], stated that the germination process can break down proteins into their constituent compounds. Substrates with higher nutrient contents can produce more methane volume [28]. Apart from nutrient contents, particle size also influenced methane production.

On the 90th d of fermentation in batch bio-digester, FGPS, either without or with grinding pretreatment, produced higher methane production than that in DGPS. This can be caused by the destruction of several vitamins due to the drying process [29] and the possibility of inactivity of the protease enzyme (resulting from the germination process) due to the drying process.

3.2. Continuous experiment

3.3. Methane production

The methane production from the co-digestion of DCM and PS using a continuous digester is shown in Table 5. Meanwhile, the evolution of methane production is shown in Fig. 3. The average methane production in the control digester (T0) was 173 L/kg VS added. This finding was in line with research by Ref. [28] reported that methane production from DCM using a CSTR digester at an HRT of 22 d and a temperature of 37 °C was 166 L/kg VS added. Meanwhile [30], reported that methane production from DCM using a plug flow digester at an HRT of 25 d and a temperature of 37–40 °C was 150–230 L/kg VS.

The results of statistical tests showed that co-digestion of DCM and PS had a significant effect ($p < 0.05$) on methane production (Table 5). Methane production from treatments T1 and T2 was not significantly different ($p > 0.05$). This can be caused by two things. First, the proportion of the VS of germinated PS to the VS of mixed substrate was relatively low (Table 1) so it cannot provide a significant increase in methane production compared to T1. Second, the grinding pretreatment reduced the positive effect of germination on methane production. Based on Tables 3 and 4, compared to the FNGPS, the FGPS can increase methane production by 180.50 % and 80.98 % for fresh and dry conditions, respectively. Then, compared to the non-germinated PS meal, the germinated PS meal increases methane production by 57.26 % and 8.04 % at fresh and dry conditions, respectively. Physical pretreatment

Table 5
Methane yield, VFA concentration, TAN concentration, VS reduction, and pH value of digested slurry.

Treatments	Methane production			VFA concentration (mM)	TAN (mg/L)	VS reduction (%)	pH
	L/L digester volume	L/kg Substrate	L/kg VS added				
T0	0.47 ± 0.77^{a}	10.32 ± 1.71^{a}	173.58 ± 28.76^{a}	48.69 ± 31.01^{b}	156.96 ± 60.90^{a}	30.00 ± 9.98^{a}	7.31 ± 0.14^{a}
T1	0.87 ± 0.17^{b}	19.13 ± 3.94^{b}	260.71 ± 53.66^{b}	5.09 ± 3.06^{a}	286.27 ± 72.38^{b}	34.30 ± 6.83^{ab}	7.54 ± 0.20^{b}
T2	0.89 ± 0.24^{b}	19.55 ± 5.27^{b}	269.92 ± 72.70^{b}	1.83 ± 0.69^{a}	257.66 ± 67.16^{b}	36.34 ± 7.87^{ab}	7.53 ± 0.19^{b}
T3	1.27 ± 0.32^{c}	27.83 ± 6.96^{c}	329.35 ± 82.32^{c}	1.93 ± 1.67^{a}	376.73 ± 104.34^{c}	38.78 ± 8.53^{b}	7.62 ± 0.19^{b}

[abc] Means in the same column without common letters are different at $p < 0.05$.

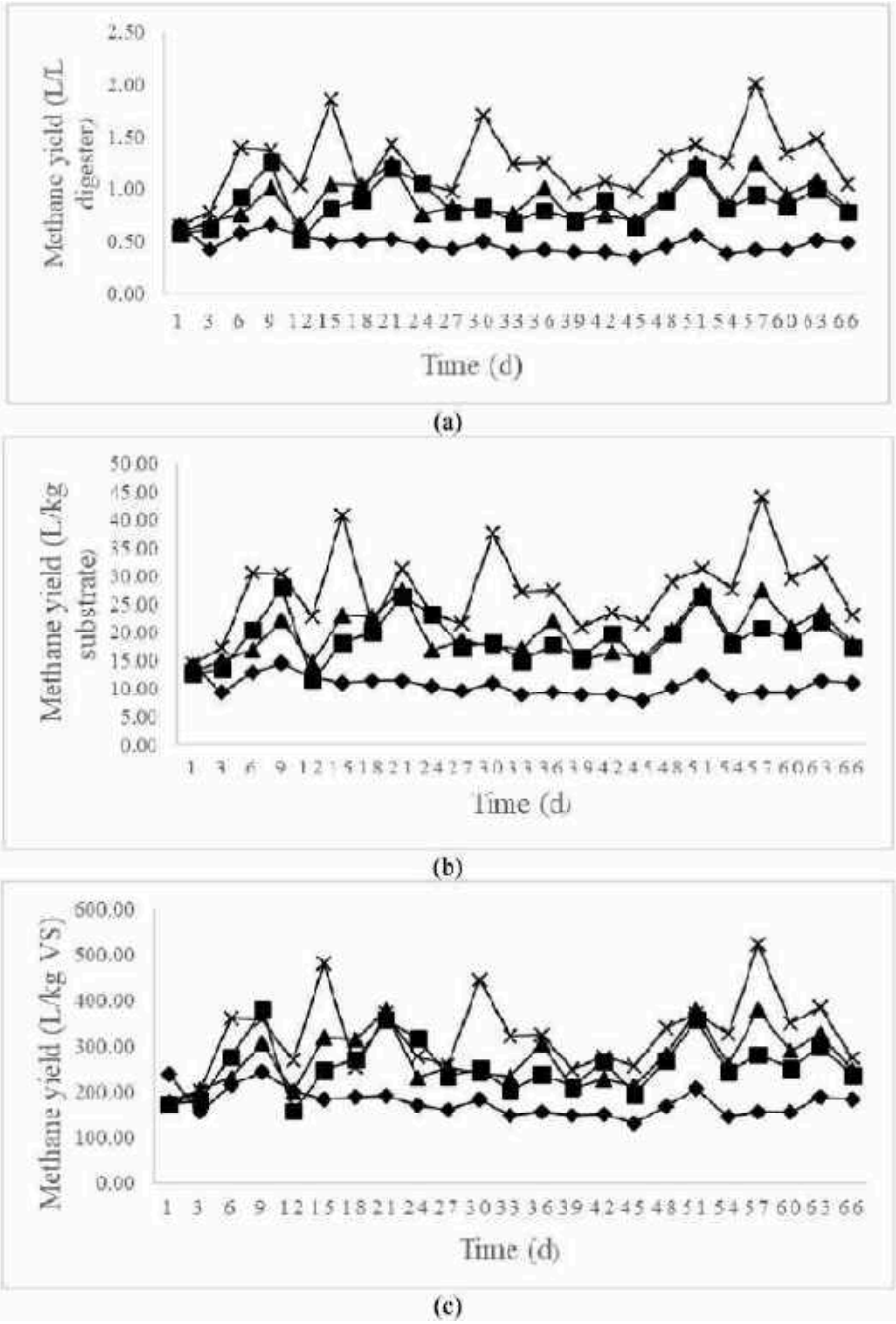

Fig. 3. Methane yield (a) L/L digester volume, (b) L/kg substrate, dan(c) L/kg VS added (◆: 100 % dairy cow manure (DCM); ■: 90 % DCM + 10 % fresh non-germinated PS meal (FNGPSM); ▲: 90 % DCM + 10 fresh germinated PS meal (FGPSM); x: 80 % DCM + 20 % fresh germinated PS meal (FGPSM)).

by reducing particle size is an effective pretreatment method because it can increase the surface area of biomass, thereby increasing its susceptibility to enzymes secreted by microbes [31]. Based on these results, compared to the FNGPSM, the FGPSM as a co-substrate with DCM resulted in a higher increase in methane. This phenomenon caused by: 1) utilization FGPSM as co-substrate with DCM can increase nutrient content in the final substrate (Table 2), 2) during germination process, endogenous protease enzymes in the PS initiate to decompose crude protein as macro nutrient in to short peptides and free amino acids [13]. Thus, the anaerobe microorganisms enable to degrade it easily and produce more methane production. Furthermore, the FGPS produced a higher increase in methane than the DGPS.

Eventhough hard to compare treatment T3 than T1 and T2 directly, since it has different level of GPS, this study found that treatment T3 resulted in the highest methane production ($p < 0.05$) compared to other treatments. It was because the treatment T3 resulted in a mixed substrate with the highest nutrient content (Table 2) compared to other treatments. In addition, the germination pretreatment can convert complex protein compounds into amino acids so it can speed up the hydrolysis stage and produce more intermediate compounds for the next stage. This was in line with the research by Ref. [13] reported that the germination process is one of the stages of growth in seeds, which can increase nutritional value, especially proteins. Seeds contain amino acids which bond with each other to form protein macromolecules making them difficult to utilize. During the germination process, there is an increase in protein degradation activity as reserves in the seeds by endogenous protease enzymes, thereby resulting in short peptides and free amino acids needed during the seed growth process. As a

consequence, the levels of peptides and free amino acids increase. They can reduce the activity of trypsin inhibitors and affect the free amino acid profile of the sprouts. The free amino acids in germinated seeds are in higher quantities than those in non-germinated seeds, so they can be used by microorganisms to produce methane [32].

A linear regression the effect utilization of FGPSM as co-substrate with DCM on methane production (L/kg VS), resulted formula $Y = 7.7886x + 179.73$. Therefore, utilization 1 % FGPSM as co-substrate with DCM resulted enhancement methane yield by 7.79 L/kg VS.

An economic analysis showed that co-digestion of PS and DCM (Table 6) showed that only treatment T1 resulted positive income, amounting to 1.94 $ USA/ton substrate when the produced electricity is connected to medium voltage and 2.59 $ USA/ton substrate if it is connected to a low voltage national electricity grid. While in other treatment, utilization of FGPSM as co-substrate with in recent study was not visible yet. However, with the increasing demand of the renewable energy, application of germination in industrial scale that can reduce the cost for germination process, and the electricity produced can be used by the company itself since the purchase price of electricity is much more expensive than the selling price. Therefore, the application of this pre-treatment is interesting to be applied in the near future.

During germination process, a waste in the form of sarcotesta layer of PS can be generated. However, this waste can be utilized as substrate in AD directly. The CO_2 and CH_4 probably can be produced during germination, nevertheless this can be neglected as emission since CO_2 produced during organic material decomposition is not calculated as greenhouse gas emission since it is part of short CO_2 cycle in the nature [34]. While CH_4 emission during this process is very unlikely to happen considering that the process is in aerobe condition, the germination is in the short period, and there is no additional starter that contain anaerobic microorganisms and naturally PS does not contain these microorganisms.

Table 6
Simple economic analysis the use of PS in co-digestion with DCM [33].

Treatments	Methane yield (m^3/ton substrate)	Δ Methane yield (N m^3/ton substrate)	Electricity production (kwh)[c]	Revenue from selling electricity (Rp)/($ USA)[d]	Net revenue from selling electricity per ton substrate ($ USA)[e]
T0	10,32	–	–	–	–
T1	19,13	8,80/8.01	27.75	29141.65/1.82[a]	1.82
				38855.53/2.43[b]	2.42
T2	19,55	9,23/8.40	29.10	30552.37/1.91[a]	−1.96
				40736.50/2.54[b]	−1.28
T3	27,83	17,51/15,93	55.21	57967.93/3.62[a]	−4.14
				77290.58/4.82[b]	−2.85

[a] And Rp. 1.400,00/kWh if it is connected to a low voltage.
[b] This study uses the currency exchange rate was a $ USA equal to Rp 15.000.00.
[c] 1 m^3 N CH_4 equal to 9.9 kwh, this paper use 35 % for power plant eficiency
[d] The Ministry of Energy and Mineral Resources, Regulation of the Minister of Energy and Mineral Resources of the Republic of Indonesia Number 27 of 2014 concerning the Purchase of Electric Power from Biogas Power Plants by PT. Perusahaan Listrik Negara is valued at Rp. 1.050,00/kWh if it is connected to medium voltage.
[e] Assuming the cost to carry out the germination process is $4/100 kg PS and no additional cost for utilization of NGPS, since the cost is charged to the waste producer.

3.4. Variables in the liquid phase

The VFA concentrations of the digested slurry at various treatments in continuous experiments using CSTR digesters are shown in Table 5. The results of statistical tests show that the co-digestion of DCM and PS had a significant effect ($p < 0.05$) on the VFA concentration. The highest VFA concentration was found at T0, namely 48.69 mM. Meanwhile, treatments T1, T2, and T3 resulted in VFA concentrations of 5.08, 1.83, and 1.93 mM, respectively.

Co-digestion of PS and DCM did not affect VFA concentrations at T1, T2, and T3, and resulted in a lower average VFA concentration compared to T0. It showed that co-digestion of PS and DCM with treatments T1-T3 improved the quality and nutritional contents compared to T0 (without PS addition). This condition can trigger the activity of anaerobic bacteria to convert VFAs into methane, so the remaining VFAs in treatment T1, T2, and T3 were lower than those in treatment T0. This was confirmed by the methane production from the three treatments (T1, T2, and T3) which was much higher ($p < 0.05$) than that in the treatment T0 (Table 5). Chow et al. [35] reported that the main advantages of anaerobic co-digestion were increases the efficiency of organic waste degradation and thereby increases biogas production, establishing an ideal nutritional balance resulting in increased anaerobe microorganisms' performance and biogas yield. In addition, co-digestion provides diversion opportunities to reduce landfill space.

Based on statistical tests, co-digestion of DCM and PS had a significant effect ($p < 0.05$) on TAN concentration (Table 5). The treatment T3 had a higher TAN concentration (376.73 mg/L) compared to the control (T0) and other treatments (T1 and T2). TAN concentrations in treatments T1 and T2 were not significantly different with average values of 286.27 and 257.66 mg/L, respectively. However, the two treatments (T1 and T2) resulted in a higher TAN concentration compared to T0 (156.96 mg/L).

The increase in TAN concentration was directly proportional to the percentage of PS addition. The proteins in PS can be degraded by microorganisms to produce ammonia. This was in accordance with the findings of [20] who reported that proteins were decomposed by microorganisms with one of the products being ammonia. Ammonia has an important role in the growth of anaerobic bacteria, but if its concentration is too high it will inhibit the methanogenesis process and even become toxic.

Co-digestion of DCM and PS had a significant effect ($p < 0.05$) on the VS reduction with the highest VS reduction obtained by T3 (Table 5). This research found that the higher the percentage of PS addition, the higher the percentage of VS reduction. The high nutrient content in a mixed substrate is thought to cause better anaerobic microorganism activity in converting organic materials into biogas. This was confirmed by that the treatment T3 resulted in a higher methane production compared to other treatments. Rajput et al. [36] stated that the higher the VS reduction reflects the higher organic material that can be digested by anaerobic microorganisms to produce higher levels of biogas.

The pH values in all treatments were still in the normal range, namely 7.31–7.62 (Table 5) so they did not interfere with the activity of methane-forming bacteria. Biogas production through an anaerobic degradation process can run well at a pH of 5.5–8.5. The optimal pH value for acidogenic bacteria ranges from 5.2 to 6.3, while it for methanogenic bacteria is in the range of 6.7–7.5. If the pH is not appropriate, the bacteria cannot live, thus having a negative impact on biogas production [37].

Based on statistical tests, the co-digestion of DCM and PS had a significant effect ($p < 0.05$) on the pH values (Table 5). The treatment T0 had the lowest pH value (7.31) of all other treatments. Meanwhile, the addition of FNGPSM and FGPSM did not have a significant effect on the pH value. The pH value tended to increase along with an increase in the percentage of PS addition. The increase in the pH value was in line with the increase in TAN concentration (Table 5). As stated by Liu et al. [38]

that ammonia has alkaline properties, therefore enhancement the TAN concentration has an impact on increasing the pH value.

4. Conclusions

Germination pretreatment was able to increase methane production from PS. The increases in methane production were 180 %, 57 %, and 81 % for FGPS, FGPSM, and DGPS. The use of FGPSM as a co-substrate with DCM was proven to be able to increase methane production 26 % higher than the use of FNGPSM. The mixed substrate of co-digestion of FGPSM and DCM had the proportion of the VS of PS to the VS of the mixed substrate was 84 % or to the weight of mixed substrate was 20 %.

CRediT authorship contribution statement

Rita Purwasih: Writing – original draft, Investigation, Formal analysis, Data curation. **Sutaryo Sutaryo:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Endang Purbowati:** Writing – review & editing, Supervision, Project administration. **Agung Purnomoadi:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

We declare that we don't have any conflict of interest.

Data availability

No data was used for the research described in the article.

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